

Impact of sodium chloride on breakfast cereal products

By

Lydie Moreau

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**The University of
Nottingham**

Division of Food Sciences
University of Nottingham, Sutton Bonington Campus
Loughborough
LE12 5RD
United Kingdom

Abstract

To reduce the amount of sodium chloride in breakfast cereals without changing their properties, it is necessary to understand the role of this salt. Hence, a model system was developed. This model, composed of native waxy maize starch, glucose and a mixture of amino-acids generated similar colour and residual volatiles after heating compared to commercial breakfast cereals. Systematically designed experiments used this model to study the influence of NaCl concentration (0 % to 5.44 %) on colour, residual volatiles and acrylamide formation. It was found that higher NaCl concentration led to darker products ($p < 0.05$) and significantly decreased acrylamide formation in the model systems. However, it did not have a significant impact on residual volatile levels. These findings were confirmed by observations made on wheat, wheat and rice mixture, corn and rice commercial cereals.

The impact of NaCl on colour and acrylamide formation indicated that this salt might influence Maillard and/or caramelisation rates and pathways. As NaCl is a plasticiser, it can allow the rubbery state to be maintained for a longer period during heating, improving reactants' mobility and Maillard reactions. However, it was found using the model systems mixed with several types of plasticisers (NaCl, KCl or trehalose), that the NaCl plasticising effect was not the major influence. The models also demonstrated that the hygroscopic behaviour of NaCl was not linked to its impact on colour and acrylamide formation.

In investigating salt's influence on starch, native waxy maize, cassava or potato starch were mixed with NaCl (0 to 4 %; moisture adjusted to 20 %) and were heated at 230 °C for 45 min. Microscopic observations, wide angle X-ray, viscosity, intrinsic viscosity and DSC data all suggested that starch was degraded by the heat treatment, and NaCl accelerated starch break down into smaller molecules, i.e. glucose. The glucose potentially formed could then caramelise, which might explain the NaCl impact on colour formation in model systems and breakfast cereals. Among other tested salts, CaCl_2 and MgCl_2 also enhanced starch degradation during a heat treatment.

In studying glucose solutions containing salt (NaCl, CaCl_2 or MgCl_2) and heated between 180 and 230 °C, it was observed that salt enhanced colour formation via caramelisation ($p < 0.05$). Mixtures of glucose, amino-acids and salt (NaCl, CaCl_2 or MgCl_2), heated under the same conditions, showed that salt significantly decreased colour formation ($p < 0.05$), which was most likely generated via Maillard reactions. Hence, salts could slow down Maillard reactions, explaining why lower acrylamide contents were found in model systems and cereal products when NaCl was present.

As NaCl seems to influence Maillard and caramelisation reactions, decreasing or removing NaCl from breakfast cereal recipes might not only alter the salty taste but also the overall flavour. CaCl_2 and MgCl_2 seemed to have similar or even more impact on colour formation compared to NaCl. Adding these salts to breakfast cereal products with a low NaCl content was found to compensate for the colour loss. Adding CaCl_2 or MgCl_2 also improved the overall flavour of breakfast cereals, even though it did not compensate entirely for the taste loss.

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List of abbreviations

- ANOVA: ANalysis Of VARiance
- APCI-MS: Atmospheric Pressure Chemical Ionisation – Mass Spectrometry
- a_w : water activity
- BBSRC CASE: Biotechnology and Biological Sciences Research Council - Collaborative Awards in Science and Engineering
- c : concentration
- CIE: Comité International d'Eclairage
- CPW: Cereal Partners Worldwide
- DMSO: DiMethyl SulfOxide
- DSC: Differential Scanning Calorimetry
- dwb: dry weight basis
- FSA: Food Standard Agency
- HMF: HydroxyMethyl Furfural
- Intersalt: International Study of Salt and Blood Pressure
- K_H : Huggins constant
- K_K : Kraemer constant
- LC-ESIMS/MS: Liquid Chromatography - ElectroSpray Ionization Mass Spectrometry / Mass Spectrometry
- M : molecular weight
- M : mol/l
- MS: Mass Spectrometry
- M_{t0} = Initial sample mass
- M_t = Sample mass at time t
- m/z : mass-to-charge ratio
- η : Viscosity
- $[\eta]$: intrinsic viscosity
- η_{inh} : inherent viscosity
- η_{red} : reduced viscosity
- η_{rel} : relative viscosity
- η_{sp} : specific viscosity
- ppm: part per million
- PTA: Phase Transition Analyser
- RH: Relative Humidity
- RVA: Rapid Visco Analyser
- SIM: Selected Ion Monitoring
- T : Temperature
- T_g : Glass Transition temperature
- TGA: ThermoGravimetric Analyser
- WAXS: Wide Angle X-ray Scattering
- ww: wet weight basis

1. Introduction

1.1. Project background

The project that forms the basis of the work reported in this thesis started in March 2006. It was conceived as a studentship awarded as a BBSRC CASE (Biotechnology and Biological Sciences Research Council - Collaborative Awards in Science and Engineering). This scheme allows a student to receive research training in collaboration with an industrial partner. Cereal Partners Worldwide (CPW) sponsored this award to look at the influence of sodium chloride (NaCl) on the quality and processing of ready-to-eat breakfast cereal products. The work was part of a global project on sodium reduction in breakfast cereals taking place within the company. CPW was formed in 1990 as a joint venture between Nestlé S.A. and General Mills. It is now the second largest breakfast cereal manufacturer in the UK, with over 25 % of a market worth more than £1.3 billion. Many well known brands are produced by CPW, such as Shredded Wheat, Shreddies, Cheerios, Cookie Crisp, Nesquik Cereals, Fitness, Clusters and Oats & More. These brands are commercialised under the name Nestlé.

1.2. Industrial relevance of the work

Sodium chloride is present in most processed foods. Today, the average NaCl daily intake in the UK has reached 9 to 12 g per person, which is twice as much as the medical recommendations (6 g per day) (He and MacGregor, 2003). Around 75 % of the sodium consumed comes from processed foods (Toldra, 2007). Therefore, food industries are encouraged to reduce the NaCl level in their products. As cereal products (mainly bread, breakfast cereals, biscuits and pastries) give rise to a third of all the sodium in foods, they are a key target for sodium reduction (Kilcast and Angus, 2007). Regarding breakfast cereals, the Food Standard Agency (FSA) in the

UK has set a target level as less than 0.8 g of NaCl (300 mg of sodium) per 100 g of product. For the time being, some commercial breakfast cereals found on the market have twice as much NaCl than this target.

The NaCl level in breakfast cereal products has decreased progressively during the last 10 years. Because this decrease was slow, the consumer did not notice any significant change in the aroma or the taste of the cereal products. Indeed, it is possible to modify someone's NaCl level preferences for a food product if this person consumes it regularly. NaCl perception rapidly adapts to low sodium diets, the consumer becoming more sensitive to lower NaCl concentrations in just a few weeks and guaranteeing a similar enjoyable taste (Toldra, 2007; Girgis et al., 2003). This technique was working until the NaCl level reached a certain perception threshold, under which any further reduction was perceived as negative by the consumer (Ferry and Hill, 2007). As the sodium content of some breakfast cereal products are still above the guidelines, modifying the recipes and/or the processes used has become essential if consumer satisfaction is to be retained.

Sodium chloride impacts on processing behaviour and several functional attributes, as well as the taste, in processed foods. This complexity represents various challenges for food manufacturers to reduce the NaCl content without food quality changes. Enhanced fundamental understanding about the impact of NaCl on food processing and on finished products would help in the development of strategies for further sodium reduction without compromising the food quality.

The main goals of this project were therefore to observe and understand the role of sodium chloride in breakfast cereal products and to find ways to compensate for a potential quality loss due to NaCl reduction.

Before presenting the results of this study, the following chapter reviews some of the current literature available with regards to the impact of a high sodium intake on health and the necessity to reduce the sodium level in processed foods. Following this, additional information is given about breakfast cereal formulation and process, plus the possible impacts of NaCl on the product. The detailed aims and objectives, as well as the thesis organization, are outlined at the end of Chapter 2.

2. Literature review

Sodium chloride has been a valuable food ingredient since the beginning of civilization, although it is not known whether it was added to food for flavour or for preservation. Its history as an additive goes back to about 3000 BC (Reddy and Marth, 1991).

NaCl consumption is not without any consequences for the human body. Indeed, sodium chloride is vital as it takes part in many body functions, but it is also one of the main factors leading to high blood pressure. Hence, its intake needs to be controlled.

2.1. Medical impact of NaCl consumption

Sodium chloride is a non-transition metallic salt required by all mammals, including humans. The sodium cation is needed to maintain blood volume and cellular osmotic pressure and is used for transmission of nerve impulses. The chloride anion is required to maintain tissue osmolarity and the acid-base balance in blood, to activate certain essential stomach enzymes and to form hydrochloric acid (HCl) in the stomach (Reddy and Marth, 1991). Hence, the consumption of NaCl is vital.

An estimate of the adult minimum daily requirements is between 0.25 and 0.5 g of NaCl. As on average, the NaCl daily intake is between 9 and 12 g/day (He and MacGregor, 2003), and from this amount 3 g is naturally present in food, the added NaCl in foods is not necessary for body functions. By contrast, this high NaCl consumption can have negative impacts on health. Indeed, evidence for an association between NaCl intake and blood pressure was provided by both observational and intervention studies (Shils et al., 1994).

Not all human beings respond equally to a high NaCl intake. The International Study of Salt and Blood Pressure (Intersalt) showed that, although individual sodium intake in most populations throughout the world exceeds the needs for the body functions, most people remain normotensive (no blood pressure change) (Adroque and Madias, 2007). In such cases, the excess sodium consumed is excreted by the kidneys within 24 h and does not affect blood pressure (Gropper et al., 2000).

Hypertension affects approximately 26 % of the adult population worldwide (Kearney et al., 2005). Among these 26 % of hypertensive people, about half are NaCl sensitive (13 % of the adult population), meaning that arterial pressure is increased by NaCl loading and decreased by NaCl depletion. The effect of dietary NaCl on blood pressure has been generally attributed to the sodium ion (Adroque and Madias, 2007; Haddy, 2006; Gropper et al., 2000).

The sodium ion influences blood pressure using several pathways in salt sensitive individuals (Adroque and Madias, 2007; Blaustein et al., 2006; Haddy, 2006; Danilczyk and Penninger, 2004; Gropper et al., 2000):

- High Na^+ ingestion is thought to increase sodium re-absorption by the kidneys. This causes water retention to maintain the plasma sodium concentration. Consequently, it increases the extra cellular fluid volume load of the body, increasing blood pressure.
- Dietary Na^+ as well as the sodium retained by the kidneys lead to an increase of the plasma level of cardiotonic steroids (ouabain or an isomer of ouabain). These molecules suppress the cardiovascular membrane $\text{Na}^+\text{-K}^+\text{-ATPase}$, resulting in reduced activity of the $\text{Na}^+\text{-K}^+$ pump. Consequently, this increases contractility of heart, arteries, and veins, hence increasing blood pressure.
- Higher local sodium concentration may facilitate calcium entry in smooth vascular muscles, elevating the blood pressure.

- Increased dietary Na^+ increases the renal excretion of potassium, resulting in a small fall in plasma potassium concentration, leading to vasoconstriction. Thus, it is possible that hypokalemia is in part responsible for hypertension.

The several ways by which NaCl affects blood pressure, as described above, are illustrated in Figure 2.1.

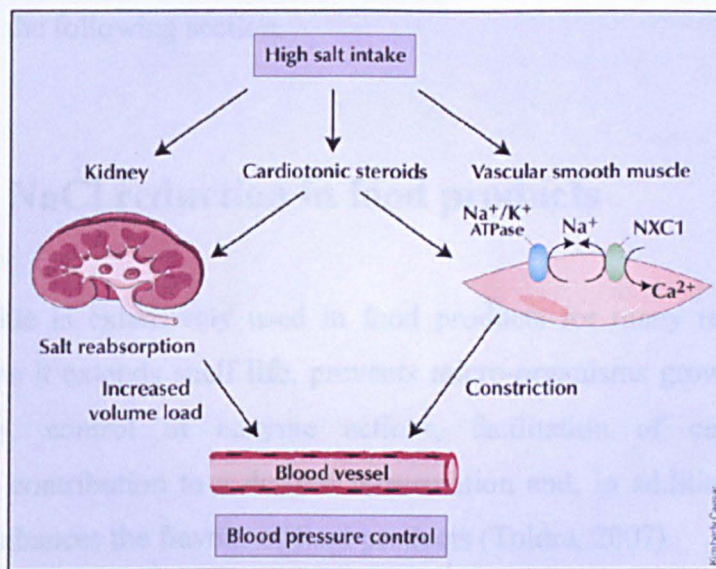


Figure 2.1: Pathways linking NaCl intake to high blood pressure in NaCl sensitive individuals (Danileczyk and Penninger, 2004).

Cardiovascular diseases (strokes, heart attacks and heart failures) are the leading causes of death and disability worldwide. Increasing blood pressure is the main cause of strokes and heart failures. It is also a very important cause of coronary heart disease (Kilcast and Angus, 2007). A study has shown that a NaCl reduction of 3 g/day would prevent 7300 to 8300 stroke deaths and 10 600 to 12 400 ischemic heart disease deaths per year in the UK. Reducing the sodium intake by 9 g/day instead of 3 g/day would triple the prevented deaths linked to high blood pressure (He and MacGregor, 2003).

Although NaCl intake can be directly linked to high blood pressure, it is important to bear in mind that these pathways do not take into account other factors (i.e. differences in body weight, in physical activity and in potassium intake). These other factors may also be responsible for high blood pressure and complicate the

assessment of the importance of NaCl intake to hypertension development (White and Crocco, 1981).

As direct correlations exist between NaCl intake and high blood pressure for the NaCl sensitive population, a reduction in sodium intake is one of the FSA's goals for improving public health. The FSA has set some guidelines for the food industries to decrease the amount of NaCl added to food products. These guidelines are highlighted in the following section.

2.2. NaCl reduction in food products

Sodium chloride is extensively used in food products for many reasons such as: preservation, as it extends shelf life, prevents micro-organisms growth and reduces water activity, control of enzyme actions, facilitation of certain proteins' solubilisation, contribution to a desired fermentation and, in addition to its typical salty taste, it enhances the flavour of food products (Toldra, 2007).

Around 75 % of the sodium intake in the UK is derived from processed foods; the rest is the natural sodium in foods and the sodium added as a condiment during cooking (Toldra, 2007).

The daily consumption of 9 to 12 g of NaCl per day is aimed to be decreased to 5 or 6 g/day. Two main strategies were taken by the FSA in 2004 to reach that objective. The first was by increasing awareness of the general public about NaCl consumption and the possible repercussions on health. This might help reduce the amount of sodium chloride added by the consumer to a food product just before eating. The second and main strategy used by the FSA to decrease the sodium daily intake was by working with food industries to encourage them to implement sodium reductions in their products.

The contribution of processed foods to sodium intake, depending on their category, is represented in Figure 2.2. Cereal and cereal products (bread, breakfast cereals,

biscuits and pastries) are the main food group contributing to sodium intake (Figure 2.2).

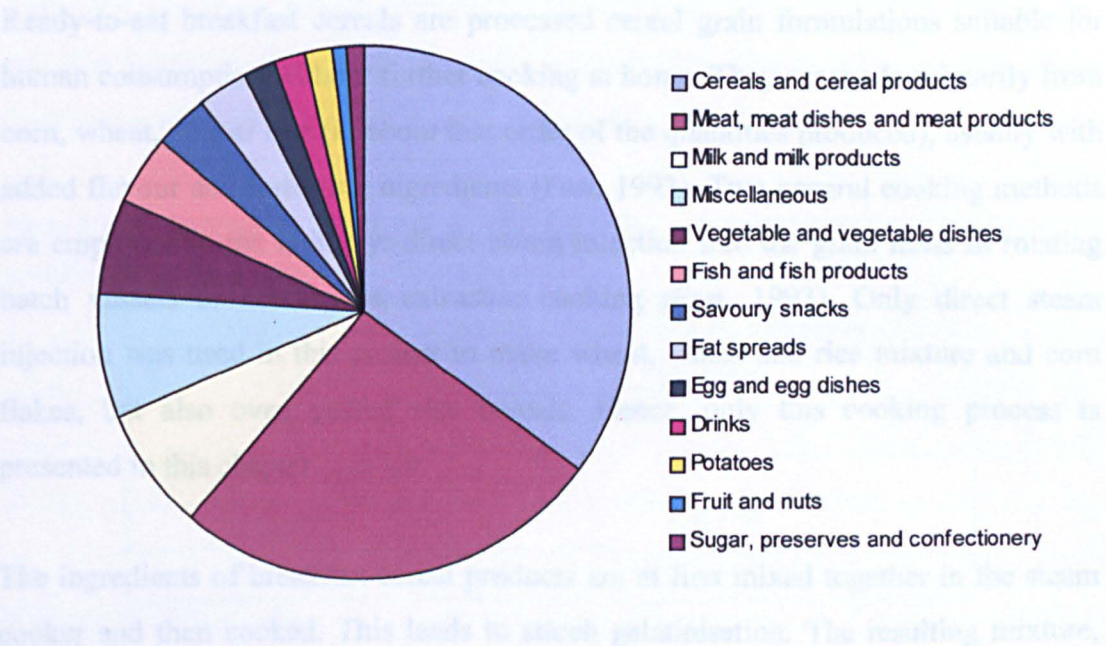


Figure 2.2: Percentage contribution of food types to average daily sodium intake (Kilcast and Angus, 2007).

To assist industries as to the type of foods in which reductions were required and the level of reductions needed, the British government developed proposals for target levels. These were published in March 2006. Regarding breakfast cereals, the target is less than 0.8 g of NaCl (300 mg of sodium) per 100 g of product. These targets will be reviewed by the FSA based on the progress made by the industries by the end of 2008 (Kilcast and Angus, 2007).

Nowadays, most breakfast cereal products contain more NaCl than this target and, for some products, the level goes up to twice the amount set by the FSA. To reach such a low level of sodium in breakfast cereal products without altering the product quality, understanding the influence of NaCl in the product is key. The following section presents the process used to make breakfast cereal products and the possible impacts of NaCl.

2.3. Ready-to-eat breakfast cereal manufacture

Ready-to-eat breakfast cereals are processed cereal grain formulations suitable for human consumption without further cooking at home. They are made primarily from corn, wheat, oats or rice (in about that order of the quantities produced), usually with added flavour and fortifying ingredients (Fast, 1993). Two general cooking methods are employed in the industry: direct steam injection into the grain mass in rotating batch vessels or continuous extrusion cooking (Fast, 1993). Only direct steam injection was used in this project to make wheat, wheat and rice mixture and corn flakes, but also oven puffed rice cereals. Hence, only this cooking process is presented in this chapter.

The ingredients of breakfast cereal products are at first mixed together in the steam cooker and then cooked. This leads to starch gelatinisation. The resulting mixture, called a dough, is dried by successive steps and then shaped into pellets, which are flattened to create flakes. The flakes are then toasted at high temperature to produce breakfast cereals. This process is presented schematically in Figure 2.3 and explained step by step in more details in the following sections.

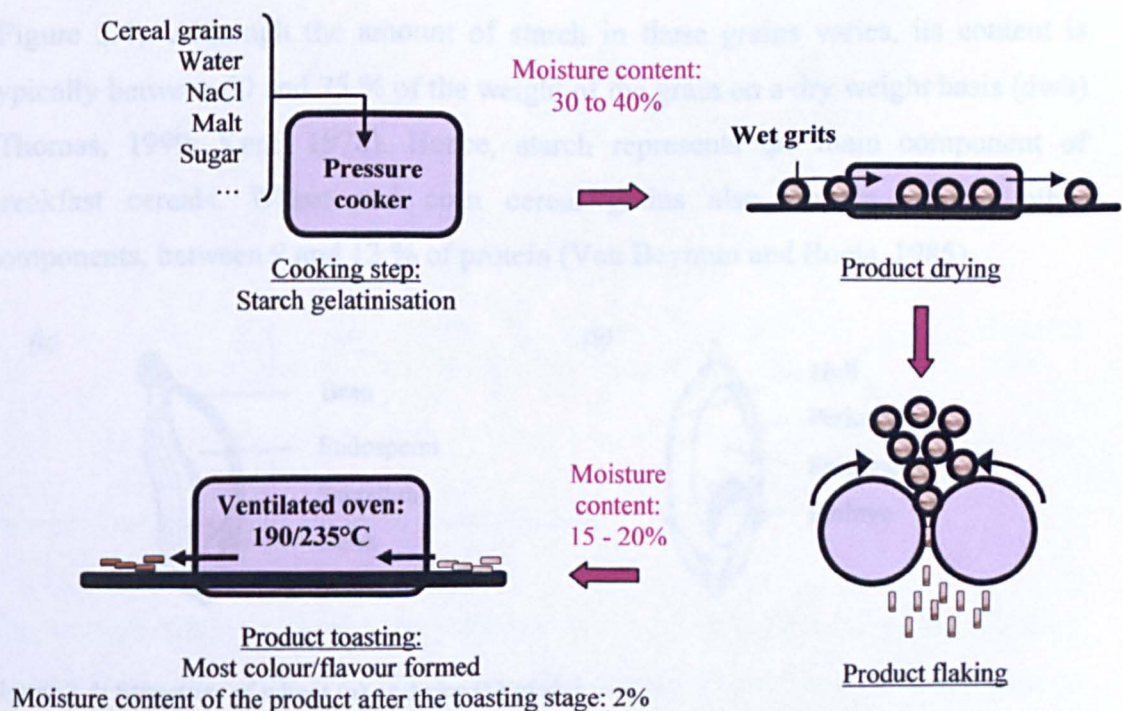


Figure 2.3: Breakfast cereal flakes process.

2.3.1. Product formulation

The main ingredients used for the production of wheat, wheat and rice mixture, corn and rice breakfast cereals are presented in Table 2.1. The moisture content of the final product is generally around 2 % wet weight basis (wwb).

Table 2.1: Formulation of final wheat, wheat and rice mixture, corn and rice ready-to-eat breakfast cereal products.

Ingredients	Relative quantity in final breakfast cereals
Cereal grains	80 to 89 %
Sugars	7 to 16 %
Water (moisture content)	2 %
NaCl	1 to 2 %
Malt syrup	0.5 to 1.5 %

These ingredients (Table 2.1) are presented successively in the following sections.

2.3.1.1. Cereal grains

Cereal grains contain mainly starch, which is located in the endosperm of the grain (Figure 2.4). Although the amount of starch in these grains varies, its content is typically between 60 and 75 % of the weight of the grain on a dry weight basis (dwb) (Thomas, 1999; Kent, 1978). Hence, starch represents the main component of breakfast cereals. Wheat and corn cereal grains also contain, among other components, between 9 and 13 % of protein (Van Beynum and Roels, 1985).

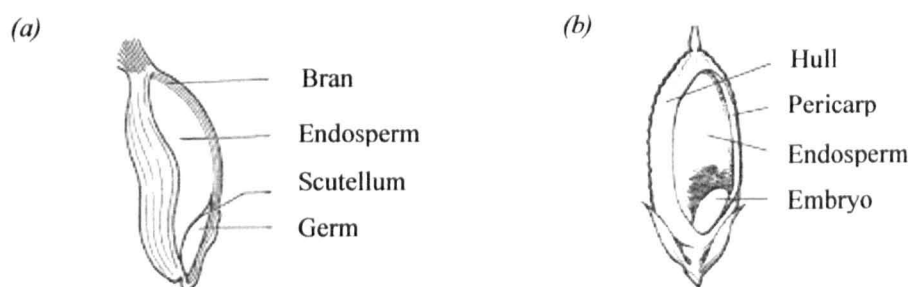


Figure 2.4: Structure of wheat (a) and rice (b) grains.

Whole grains were used in breakfast cereal products, but before entering the formulation, the cereal grains were transformed. The corn and wheat grains were “cracked”, which means that they were broken into smaller pieces. The size obtained was about one half to one third that of the whole kernel. Hence, the integrity of the grain and its endosperm was altered. The starch granules contained inside the endosperm were directly in contact with the other ingredients of the formulation during the mixing step. Regarding the rice grains, the hull was removed (Figure 2.4).

As previously mentioned, starch is the main component of cereal grains. It consists primarily of two types of D-glucopyranose polymers, amylose and amylopectin. The amylose / amylopectin ratio depends on the botanical origin of the starch and corresponds on average to the following: 28 / 72 for wheat and corn, and 17 / 83 for rice. Waxy maize starch contains almost only amylopectin molecules (Van Beynum and Roels, 1985).

Amylose is an essentially linear polymer composed of α -1,4-linked D-glucopyranose molecules. Amylopectin is a much larger and branched D-glucopyranose polymer containing both α -1,4 and α -1,6 linkages. The α -1,6 linkage represents the bond at the polymeric branch point (Figure 2.5).

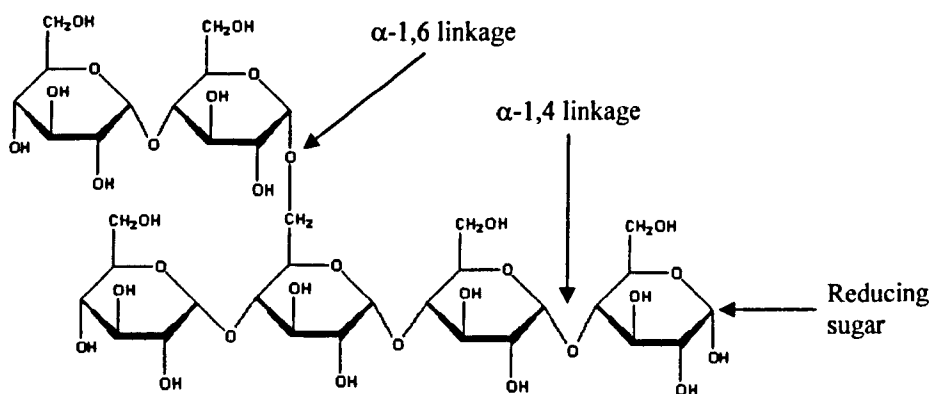


Figure 2.5: α -1,4 and α -1,6 glycosidic bonds of starch.

The reactive aldehyde group at the carbon number 1 on a D-glucose molecule makes it a reducing sugar (Thomas, 1999). A reducing sugar is any sugar that can form an aldehyde (or a ketone). When the aldehyde group of the sugar is free, i.e. when it is not locked into the ring structure or not linked to another molecule, the aldehyde can

react with some other compounds. This is the case in Maillard reactions, which is presented in section 2.4.2.1. For both amylose and amylopectin, as the aldehyde group on one end of the starch polymer is always free, the starch molecule always has one reducing end group as shown in Figure 2.5 (Thomas, 1999).

Amylose and amylopectin do not exist free in nature but form a dense, water-insoluble and partially crystalline granule. The starch granule size ranges from about 1 to 100 μm in diameter, depending on its botanical origin. Some proteins are located at the surface of the starch granule in association with other minor granule components (such as lipids). In starch granules, proteins represent between 0.05 and 0.25 % of the granules in weight, and lipids between 0.05 and 1.0 %, depending on their botanical origin (Baldwin, 2001).

A native starch granule is a semi-crystalline structure. Indeed, the branched structure of amylopectin inside a native starch granule generates alternating crystalline and amorphous regions (Figure 2.6).

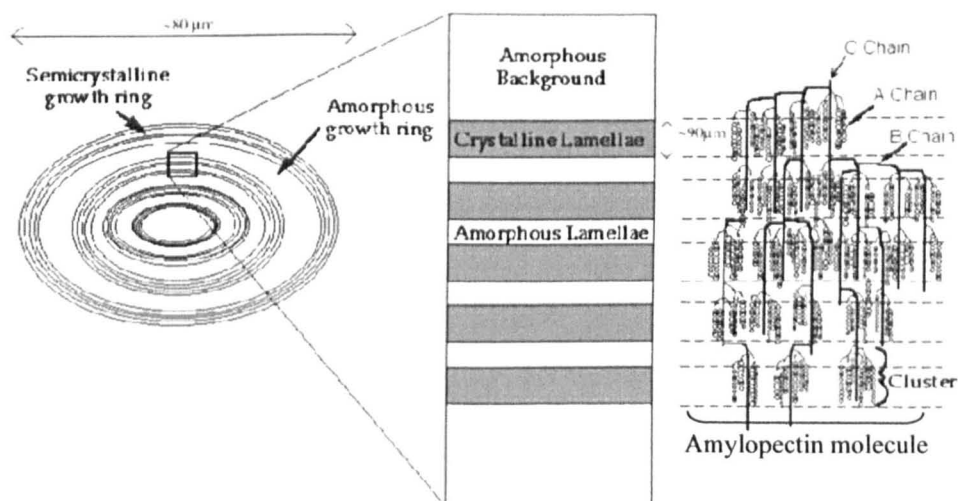


Figure 2.6: Schematic view of the structure of a starch granule, with alternating amorphous and semi-crystalline zones constituting the growth rings. Adapted from Jenkins et al. (1993).

The chains of the amylopectin polymer can associate to form double helices and create the crystalline part of the granule. The combinations of amylose and the amylopectin create densely packed crystalline blocks and less dense amorphous regions. On a larger scale, these lamellae of crystalline and amorphous bands are

grouped into structures known as growth rings. Between these growth rings are amorphous zones (Figure 2.6).

After cereal grains (hence starch), sucrose and sugar syrups are the second main ingredients of breakfast cereals (weight-wise).

2.3.1.2. Sugars

Sugars, among them sucrose and sugar syrups, are essentially used for the sweet notes they give to breakfast cereals and to improve the sensory attributes of the products. Indeed, the sugars can caramelize or react further via Maillard reactions to generate colour and flavours, as presented in section 2.4.2.1. In the case of sucrose, to be able to participate to Maillard reactions, a heat treatment above 70 °C is necessary to break down this sugar into the reducing sugars glucose and fructose (Davies and Labuza, 1997). Presence of sugars also influences the texture of the product during the process. It increases the stickiness of the cooked cereal grains, which is essential to process breakfast cereals.

2.3.1.3. Malt

Malt syrups are used to add flavour to breakfast cereals. Care has to be taken to use nondiastatic malt, meaning that this syrup does not retain its enzyme activity (α - and β -amylase). Otherwise, the presence of these active enzymes might liquefy the starchy components of breakfast cereals.

2.3.1.4. NaCl

NaCl is primarily used for the salty taste it gives to the breakfast cereal products. Originally, it was thought that the saltiness of NaCl stemmed from the chloride anions. However, recent evidence suggests that saltiness is produced by sodium cations. As well as a salty taste, NaCl enhances the other flavours present in the product (Reddy and Marth, 1991). The presence of NaCl has also many other repercussions on the products, as will be presented throughout this thesis.

2.3.2. Ingredient mixing

2.3.2.1. Process specifications

The first step in making breakfast cereal products is to mix the water soluble ingredients (such as sugars, NaCl and malt), presented above in Table 2.1, with water (Figure 2.7). The resulting liquor contains around 50 % water. This liquor is then added to the cereal grains (and possibly to other non water soluble ingredients) already present in the steam cooker. The water content of the mixture is then between 11 and 18 % (wwb) before the steam cooking step, which will increase the mix water content between 30 to 40 % (wwb) (section 2.3.3).



Figure 2.7: Dissolution of the water soluble ingredients of ready-to-eat breakfast cereals in water, before their addition to the cereal grains.

Once all the ingredients are in the steam cooker, it is hermetically sealed. The cooker then starts to rotate to ensure that the solution of sugar, NaCl and malt is homogeneously blended with the cereal grains. Some steam is then injected in the cooker to start the cooking process, as presented in section 2.3.3.

2.3.2.2. Influence of NaCl during the ingredient mixing step

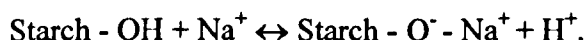
At this stage of the process, before injecting the steam, the sodium and chloride ions are in an aqueous solution. As the cooker starts to rotate, the starch granules, coming

from the damaged cereal grains, are in direct contact with the other ingredients of the formulation.

- NaCl and starch granule interactions

The interactions occurring between the starch polymer and some electrolytes such as NaCl reported in the literature remain ambiguous as contradictory results were obtained by different authors, such as follow.

Literature reports that electrolytes, like the sodium and the chloride ions, can interact with the starch granules and the starch molecules in an aqueous solution. Indeed, when starch granules are placed in water, they should be freely penetrated by water and by small molecules up to a molecular weight of about 1,000 g/mol (Hoseney, 1986). According to Oosten (1983), starch can adsorb cations from a solution in exchange with hydrogen ions, utilizing the weak ion exchanging properties of starch, forming alcoholate functions:



Hence, exchanges with bigger cations could stretch the starch matrix, increasing the volume of the starch granule (Oosten, 1983). Rendleman (1978) also proposed that amylose could form alcoholates with cations, but only in an alkaline environment. This author mentioned that the ability of a given metal salt to associate with a neutral carbohydrate ligand could increase with increasing cationic radius: $\text{Li}^+ \ll \text{Na}^+ < \text{K}^+, \text{Cs}^+$.

Some interactions between starch and cations were reported by other authors, even though the formation of alcoholate remains controversial. Chinachoti et al. (1991) showed that when starch gelatinises, the mobility of sodium ions decreases because of salt–starch interactions. Ma et al. (2007) observed that Na^+ could complex with polar groups in starch like the oxygen of the C-O-C and C-O-H groups. Ciesielski et al. (2003) demonstrated that gelatinised starch forms high spin complexes with metal cations such as Na^+ when salts and starch are mixed with water before drying. Different affinities among metal ions to a given ligand (i.e. different botanical origin

of the starch) were observed. Lai et al. (2001) reported that it does not seem clear whether salts form coordination compounds, either by involvement of lone electron pair orbitals of oxygen atoms from the glucose units of starch, or if cations are bound electro-statically to their complementary anions that are in turn bound to the hydroxyl groups of starch.

In many cases, the interactions between salts and starch seem to be more precisely between the cations and the starch molecules. Oosten (1990) explained that when pure starch is suspended in pure water, the original H^+ concentration in the water phase is five times lower than inside the starch granules. Hence, the hydrogen ions migrate into the water phase, due to the concentration gradient. But, as soon as the first hydrogen ion has left the starch particle, the latter is left with a negative electrical charge, which ultimately prevents the migration of hydrogen ions from the starch into the water phase. The resulting potential between starch and water is called the Donnan potential. When sodium chloride is added to this suspension of starch granules in water, the chloride ions are repelled from the starch particle by the negative Donnan potential. On the other hand, the sodium ions tend to penetrate the starch particle as these positively charged cations are attracted by the negative Donnan potential. Moreover, the concentration gradient tends to push these cations into the starch particles, replacing there a number of hydrogen ions, which move to the water phase (Oosten, 1990). This theory is however not in accordance with that reported Lii et al. (2002) and Ciesielski et al. (2003), who stated that the interactions between electrolytes (such as the one of the first non-transition group like NaCl) and starch granules involve anions rather than cations.

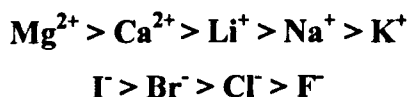
Numerous studies on starch interactions with metal salt solutions resulted in ambiguous conclusions. The exact nature of the interactions between salts and starch, and whether salts penetrate into the starch granule interior or absorb on the granule surface remain unclear (Szymonska et al., 2008; Ciesielski et al., 2003; Lii et al., 2002; Lai et al., 2001; Bircan and Barringer, 1998; Tomasik and Schilling, 1998). Even though the nature and the extent of the interactions that could arise between the NaCl and the starch granules during the ingredient mixing step are controversial, they could have some repercussions on the final breakfast cereal products.

- The Hofmeister series

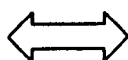
The sodium and chloride ions could also interact with the proteins present in the cereal grains during the ingredient mixing step. Indeed, in the 19th century, it was discovered that various salts at high concentrations could precipitate proteins (Kunz et al., 2004; Hofmeister, 1888). This salting-out effect has since been found to be a very general phenomenon, occurring not only on proteins but also on amino acids and even simple gas molecules. An increase in solubility at low salt concentrations followed by a decrease in solubility at high salt concentrations was observed (Zhou, 2005; Baldwin, 1996). The salting-out efficiency has a distinct order with respect to different types of salts. This order is known as the Hofmeister series.

The Hofmeister (or lyotropic) series rank the relative influence of ions on the physical behaviour of a wide variety of aqueous processes ranging from colloidal assembly to protein folding (Zhang and Cremer, 2006). Originally, it was thought that an ion's influence on macromolecular properties was caused by 'making' or 'breaking' bulk water structure. Structure-makers (or kosmotropes) and structure-breakers (or chaotropes) are terms referring to an ion's ability to alter the hydrogen bonding network of water. The kosmotropes are strongly hydrated and have stabilizing and salting-out effects on proteins and macromolecules. Chaotropes are known to give rise to salting-in behaviour (increase in solubility) (Curtis et al., 2002; Hribar et al., 2002). However, some studies demonstrated that the bulk water structure is not central to the Hofmeister effect. Instead, models are being developed that depend upon direct ion-macromolecule interactions as well as interactions with water molecules in the first hydration shell of the macromolecule (Zhang and Cremer, 2006).

The Hofmeister classification of some ions is as follows. This order might differ slightly depending on the literature as there are variations with the protein type, the system pH and temperature (Curtis et al., 2002; Cacace et al., 1997):



Weakly hydrated ions
Structure breaker (chaotropes)



Strongly hydrated ions
Structure maker (kosmotropes)

Among the macromolecules, not only the proteins could be affected by salts depending on their position in the lyotropic series. Indeed, it was reported that the gelatinisation of starch in the presence of salts depended also on the Hofmeister series (see section 2.3.3.2). It was previously mentioned that the interactions between salts and starch remain unclear. These interactions could be influenced by the anions and cations' positions in the Hofmeister series and could modify the breakfast cereal properties, as presented in the result chapter 7.

After mixing the breakfast cereal ingredients in the cooker as explained previously, some steam is injected to cook the product. This step is presented in the following section.

2.3.3. Cooking

2.3.3.1. Process specifications

Cooking is a necessary step in every process by which ready-to-eat breakfast cereals are produced. In addition to the development of some desirable flavour created via Maillard reactions (section 2.4.2.1), the cooking step creates the physical properties necessary for the development of the product texture, primarily by gelatinisation of the starchy grains (Fast, 1993). During the cooking of cereal grains, they absorb heat and moisture, and undergo physicochemical changes, such as starch gelatinisation. This phenomenon is also indispensable to make the product edible.

Starch gelatinisation is the collapse (disruption) of molecular orders within the starch granule. This is manifested by irreversible changes in their properties such as granular swelling, native crystalline melting, loss of birefringence and starch solubilisation (Thomas, 1999). The initial granule swelling takes place in the amorphous regions of the granule, disrupting the weak bonding between the starch molecules and hydrating them. As the temperature of the starch mixture rises, more hydration occurs in the amorphous regions and the hydrogen bonds in the crystalline regions begin to be disrupted. The granules continue to expand to a greatly reticulated network. A portion of the amylose molecules then leach out into the

aqueous substrate (Van Beynum and Roels, 1985). The viscosity increase that occurs when starch is heated in excess water is the result of the starch taking up water and of the substantial granule swelling. This phenomenon is called starch pasting.

During the cooking step of breakfast cereal products, the ingredients are mixed continuously by the rotation of the vessel on its axis, and heated under pressure ($\cong 1.2$ bars) at 120 °C with steam between 1 and 2 h depending on the product. The steam contacts the grains directly. The moisture content of the product at the end of the cooking step reaches 30 % (wwb).

The starch gelatinisation temperature varies depending on the botanical origin of the starch and on the process conditions (pressure, moisture content, pH, solid content...) (Thomas, 1999; Van Beynum and Roels, 1985). However, under the severe conditions applied during the breakfast cereal cooking step, it can be assumed that the starch present in the product is fully gelatinised (Whistler et al., 1984).

After the cooking step, the product obtained is called a dough. Although it is not a proper dough as there is no gluten network (the cereal grains can be separated from each other), the grains stick together when the product comes out of the cooker (Figure 2.8). The fact that the cereal grains stick together after the cooking step is mainly due to the presence of sugars, which coat the cereal grains, and the gelatinised starch, which became soluble during the cooking stage and also coats the grains.



Figure 2.8: Breakfast cereal dough coming out of the steam cooker.

2.3.3.2. *Influence of NaCl on starch gelatinisation*

Depending on its concentration, NaCl can cause either an elevation or a depression of the gelatinisation temperature of starch, as observed by several authors (Salvador et al., 2003; Chiotelli et al., 2002; bd Ghani et al., 1999; Evans and Haisman, 1982; Oosten, 1982; Wootton and Bamunuarachchi, 1980). Indeed, the gelatinisation temperatures of wheat starch measured in sodium chloride solutions were higher than that obtained in pure water for NaCl concentrations between 0 and 7 % (g of NaCl / 100 g of starch-water mixture). However, when the NaCl concentration exceeded 7 %, the gelatinisation temperature decreased as the concentration increased.

One of the major effects of adding a solute to a starch suspension might be the competition between the starch and the solute for water. It may reduce the amount of water available for the gelatinisation process which might lead to an increase of the gelatinisation temperature (Chiotelli et al., 2002). However, such a hypothesis may not explain the gelatinisation temperature decrease above a certain NaCl concentration.

Rumpold and Knorr (2005) reported that the capability of salts to influence starch gelatinisation could be attributed to structure-making and structure-breaking effects of the salts on water (Hofmeister series, see section 2.3.2.2). The highly structured aqueous solutions (with structure maker salts) might retard the diffusion of water molecules into the starch granules and increase the gelatinisation temperature. Reverse influence of the structure breaker salts might occur (Oosten, 1982). In addition, the strong electrostatic interactions between the structure maker salts and the water increase the viscosity of the solution, which in turn decrease the diffusion rate of the salt solution into the starch granules (Koch and Jane, 2000). Oosten (1982) added that the salts' influence on starch gelatinisation might as well be due to electrostatic interactions between the salts and the hydroxyl groups of starch.

It was observed that when wheat grains are boiled in salty (with NaCl), rather than fresh water, moisture uptake seemed to be inhibited. This might be due to the water structure making ability of NaCl, which reduces the overall driving force for

moisture uptake (Horrobin et al., 2003). Chiotelli et al. (2002) suggested that NaCl may strengthen the structure of water or improve starch-water interactions, but above a concentration of 7 %, it seems to rather behave like a water structure breaker.

Another explanation could be that when NaCl is added to a starch suspension, some protons of the starch alcohol groups are replaced by the sodium cations (the starch behaving like a weak acid ion exchanger, as presented in section 2.3.2.2). The sodium cations might then protect the starch granule by exclusion of the anions from the granule via the Donnan Potential. This may then change the starch granule properties and increase the gelatinisation temperature up to a certain NaCl concentration (Oosten, 1982). Above this concentration, the influence of the chloride anions may become dominant. They might enter the starch granules and start the gelatinisation by rupturing the hydrogen bonds between the starch molecules, decreasing the gelatinisation temperature at high NaCl concentrations (Oosten, 1983; Oosten, 1982).

Hence, according to these studies, the level of NaCl in breakfast cereal products might affect the gelatinisation temperature of the starch, which may modify the product properties.

After the cooking step, the obtained dough is a sticky mass of grains. In order to further process the dough, the lumps need to be broken, as explained in the following.

2.3.4. Lump breaking and pellet formation

2.3.4.1. Process specifications

The masses of cooked grains, when dumped from the batch cooker, are usually much bigger than the desired finished cereal pieces (Figure 2.9). Hence, lump breaking is an essential step for obtaining smaller and uniform pieces of cereal product that can be processed further. The lump breaking process is performed on the hot, sticky mass of cooked grains and other ingredients, just after the cooking step. Indeed, at that

stage, the cereal grains are stuck together (with the help of the sucrose, sugar syrups and the gelatinised starch coating the grains) but still can be separated from each other as the dough is soft. If the product is too cold while this process step is performed, the cereal grains would be hard, due to the starch retrogradation. Starch retrogradation is a process which occurs when starch chains begin to re-associate in an ordered structure. In its initial phase, two or more starch chains may form a simple juncture point which then may develop into more extensively ordered regions. Ultimately, a crystalline order appears (Thomas, 1999). Such retrogradation would cause hardening of the dough, making this product not processable anymore.

During the lump breaking process, the mass of grain is passed through a rotational machine, where its mechanical action separates the sticky cereal pieces from each other (Figure 2.9).

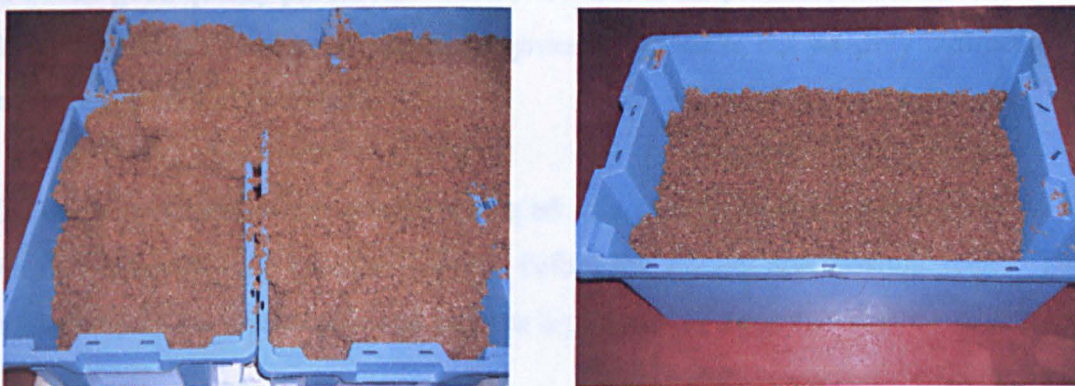


Figure 2.9: Breakfast cereal dough before (left picture) and after (right picture) the lump breaking process.

After the lump breaking step, the cereal grains from the dough are separated from each other, but still have a sticky behaviour. Hence, they can be re-associated into pellets if mechanical forces are applied to the product. In order to have breakfast cereal pellets of a certain length, width and thickness, the dough is pressed mechanically and then cut with regular dimensions.

As for the lump breaking process, the pellet formation has to be performed on a hot and sticky mass of cooked grains for the grains to retain the shape of pellets after such a treatment. If the mass was too cold, the grains would separate from each

other. Lump breaking and pellet formation steps are not necessary for the production of puffed rice.

2.3.4.2. Influence of NaCl during lump-breaking and pellet formation steps

It was previously mentioned that the presence of NaCl can influence starch gelatinisation. Therefore, the dough stickiness may be influenced by the level of NaCl in the product. If the dough is too sticky, jam-ups could occur during the lump-breaking process. In such cases, the lump breaker then would turn into a powerful mixer that destroys the product rather than performing its breaking function. By contrast, if the dough is not sticky enough, the cereal grain could not hold together in the shape of a pellet. Hence, the control of the dough texture (stickiness) is essential to obtain good quality products. Presence of NaCl in the product should not influence the lump-breaking and pellet formation processes as such, but possibly indirectly by influencing the dough texture.

Once the breakfast cereal dough is shaped into pellets, they are dried in order to obtain the appropriate moisture content before the flaking and toasting stages. The drying step of the breakfast cereal process is presented below.

2.3.5. Drying

A controlled removal of water from the breakfast cereal pellets is performed to obtain the appropriate pellet physical properties. Indeed, the moisture content of the product after drying is one of the most important parameter which determines the further processability of the pellets. If the moisture of the pellets is too high during the flaking process and the centre of the pellets is still too gummy, the flakes would ball up on the flaking roll knives (Fast, 1993).

During the drying step, the product is placed on perforated metallic trays (product layer thickness of around 5 cm), allowing the air to circulate through the product.

The air temperature is between 50 and 90 °C, depending on the product, with a gas phase moisture content of 50 %. The product dries between 10 and 30 min, the pellets are then moved around before another drying period of 10 to 15 min. The moisture of the product drops from around 30 % to 15 - 20 % (wwb) during the entire drying step.

After this drying step, a tempering stage takes place, where the product is left for at least 2 hours to obtain a more uniform moisture content within and among the cereal particles, even though a moisture gradient remains. Some processes include overnight tempering. Such tempering times allow the starch to retrograde (section 2.3.4.1). It increases the firmness of the grains due to starch retrogradation, giving products of higher quality during the flaking process (Fast, 1993).

2.3.6. Flaking

Flaking mills consist of two rolls, one rotating clockwise and the other anticlockwise. The pellets are passed between these two rolls to flatten them into flakes. One of the roll is adjustable so that the distance between them can be set to produce flakes of the desired thickness (Figure 2.10).

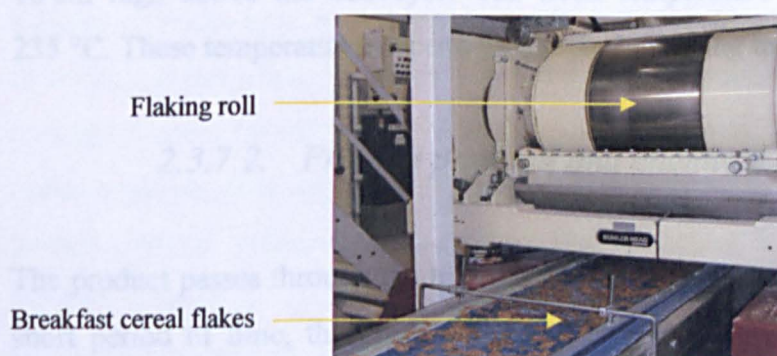


Figure 2.10: Flaking rolls used to transform breakfast cereal pellets into flakes.

These flaking rolls are hollow to allow water to pass through them to cool down their surfaces. This cooling system is necessary to control the flake temperature and optimise the quality of the products.

For the needs of the present study, oven puffed rice breakfast cereals were produced as well as wheat, wheat and rice mixture and corn flakes. The process of the oven puffed rice differed from the flakes during the flaking step. They were bumped by running them through the flaking rolls to slightly flatten them, but not as much as the flakes (1.4 mm instead of 0.6 mm). Bumping presumably creates fissures in the kernel structure, which promote expansion (puffing) at high oven temperatures during the toasting stage (Fast, 1993). The rice grains were then toasted like the flakes. This toasting treatment is presented in the next section.

2.3.7. Toasting

This last operation is one of the most important as it gives the product its texture, colour, aroma and taste.

2.3.7.1. Process specifications

The unbaked, shaped flakes are placed on a conveyor band. The conveyor carries the flakes through an oven chamber from one end to the other. In order to have uniformly toasted flakes, hot air circulates so that the flakes are floating at about 10 cm high above the conveyor. The oven temperature varies between 180 and 235 °C. These temperatures depend on the product being toasted.

2.3.7.2. Product physical and chemical changes

The product passes through the toasting tunnel in less than a minute, but during this short period of time, the product acquires its characteristic properties due to this severe heat treatment:

- The product dries and passes from a rubbery to a glassy state.
- Two types of chemical reactions (named Maillard and caramelisation reactions) generating colour, aroma and flavour, are favoured during the toasting step and give to the product its characteristic taste and colour.

- The integrity of the starch molecule can be affected.

These product modifications are detailed one by one in the following sections. Presence of NaCl can have an impact on each of these product modifications. A review of the possible impacts of NaCl is also given in the following sections.

It should be noted that during the toasting step of breakfast cereals, air bubbles are formed. Indeed, the application of high temperatures causes the product to expand, hence the formation of bubbles. Those are contributing to the overall appearance and texture of the food product.

2.4. Product changes during the toasting step and influence of NaCl

2.4.1. Drying and state transition

The flakes dry, from 15 – 20 % moisture at the entrance of the toasting tunnel to 2 % moisture in the finished product. The state of the product changes during this heat treatment. When the product enters the toasting tunnel, it is considered to be in a rubbery state due to its high moisture content. As it dries during the toasting step and then cools down when the flakes exit the toasting tunnel, the breakfast cereal flakes undergo a glass transition and they enter a glassy state. The consumer finds food under a glassy state appealing for a number of reasons, but particularly for their texture. Indeed, crispiness and crunchiness are linked to the glassy state of the product (Figure 2.11) (Pittia and Sacchetti, 2008; Gondek and Lewicki, 2006; Martinez-Navarrete et al., 2004; Pamies et al., 2000; Le Meste et al., 1996; Roos, 1995). This transition is therefore an important stage to control during the process.

Glass transition is the name given to the phenomena observed when a liquid or a rubbery product is cooled sufficiently quickly below its equilibrium crystalline melting temperature to avoid its crystallisation. This solidification process results in the immobilisation of the disordered structure (non-crystalline state) (Slade and

Levine, 1993). The long range cooperative motions are then restricted and the product becomes a glass. Motions (vibrations of atoms, reorientation of small groups within a molecule) are then mainly local and are not involving the surrounding atoms or molecules, as occurs in the rubbery material. This decrease in molecular mobility parallels a sudden increase in viscosity. Hence, a glass is described as a liquid whose viscosity is greater than 10^{12} to 10^{13} P that can support its own weight (does not flow). The temperature at which the product changes from a rubbery state to a glassy state is called the glass transition temperature (T_g) (Le Meste et al., 2002; Biliaderis et al., 1999; Roos, 1995; White and Cakebread, 1966). This is what happens to breakfast cereals during the toasting step.

In the case of breakfast cereals, the wet flakes are visco-elastic (or rubbery) as they are a polymeric material. When the rubbery wet breakfast cereal flakes are toasted, water is removed quickly from the product. The removal of water from the product during the toasting step is the main cause of the product entering the glassy state. Indeed, many studies have shown that water exerts a strong plasticising action in hydrophilic polymers such as starch (Biliaderis et al., 1999).

A plasticiser is a molecule, which when added to a system, decreases the glass transition temperature of this system. The effect of plasticiser molecules on polymers can be explained in terms of two mechanisms (Gondek and Lewicki, 2006):

- The plasticisers might screen off attractive forces between polymer chains
- The plasticiser molecules may enlarge the spaces between polymer chains allowing chain segments greater freedom of movement.

On a molecular level, moisture induced plasticisation of a polymer leads to increased molecular distances (free volume), decreased local viscosity and increased back-bone chain segmental mobility. The addition of a low molecular weight plasticiser (such as water or NaCl) to an amorphous matrix has the same effect as a temperature increase on molecular mobility (Le Meste et al., 2002).

The presence of water in wet breakfast cereal flakes before the toasting step leads to a T_g below 25 °C as water is a plasticiser. This is why these wet flakes are under a

rubbery state before the heat treatment. The removal of water from the product during the heat treatment decreases the free volume and the chain mobility and increases local viscosity. This leads to an increased T_g (well above ambient temperature). When the breakfast cereal product exits from the toasting tunnel, it has a high temperature and the flexibility of the flakes indicates that it is under a rubbery state. As it rapidly cools down in contact with the air at 25 °C, it enters the glassy state as the T_g of the product is above ambient temperature (due to its low moisture content).

The change in molecular mobility due to the physical state of a product leads to many other physical properties of the system, as presented in Figure 2.11.

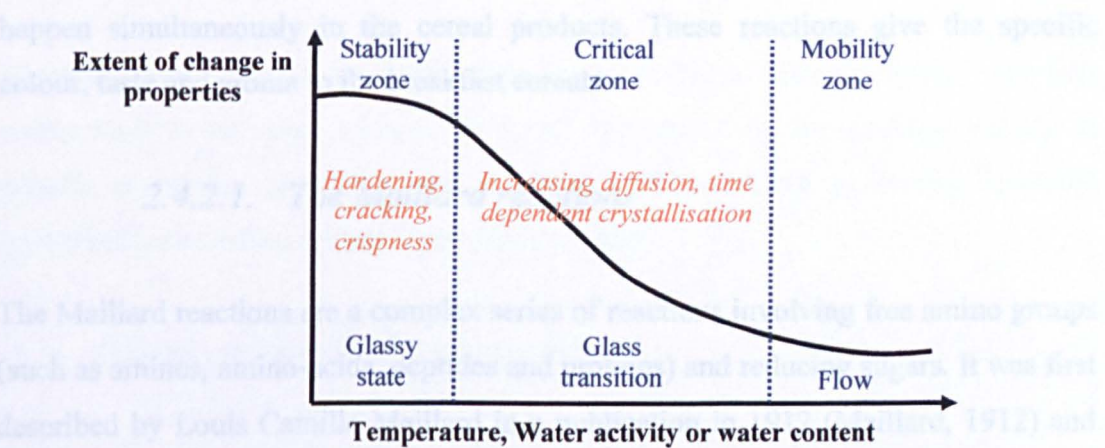


Figure 2.11: Effect of temperature, water activity or water content on relative rates of mechanical changes of amorphous biological materials (Roos, 2003).

The glassy state is frequently described as a state of relatively high stability (Figure 2.11). Diffusion controlled physical or chemical processes practically cease and only localised small amplitude motions continue to occur. This is why Maillard reactions, which involve the reaction of two types of molecule, can be affected by the physical state of the product (see section 2.4.2.1) (Le Meste et al., 2002). NaCl being a small molecular weight compound, it might act as a plasticiser in breakfast cereal products, like water. NaCl was previously reported to decrease the glass transition temperature of starchy products (Laaksonen and Roos, 2003). Presence of NaCl in the recipe might decrease the temperature at which the flakes enter a glassy state when they exit from the toasting tunnel. Hence, the flakes might remain in a rubbery state longer

compared to a product without NaCl in its composition. This might have some consequences on the extent of some reactions.

2.4.2. Flavour, aroma and colour formation

Heating a food product in a dry environment significantly enhances the flavour and colour of foods, and these changes are generally related to non-enzymic browning during heating (Ledl and Schleicher, 1990). During the toasting step, as high temperatures are applied to this intermediate to low moisture product containing sugars and proteins, Maillard and caramelisation reactions are favoured. They can happen simultaneously in the cereal products. These reactions give the specific colour, taste and aroma to the breakfast cereals.

2.4.2.1. *The Maillard reactions*

The Maillard reactions are a complex series of reactions involving free amino groups (such as amines, amino-acids, peptides and proteins) and reducing sugars. It was first described by Louis Camille Maillard in a publication in 1912 (Maillard, 1912) and was ignored by the scientific community until 1941. In 1948 the Maillard reactions were definitely recognized as being responsible for the browning and loss of nutritive value of heated milk powders. There was then a continuous increase in papers on the chemistry of this complex reaction to identify its various pathways. Hodge presented the first comprehensive reaction scheme showing the complexity of the Maillard reactions chemistry (Hodge, 1953). Forty years later, his scheme was still and remains nowadays the basis for current understanding of the essential features of the reaction (Mottram, 1994).

Maillard reactions influence the organoleptic characteristics of many food products. Indeed, during a heat treatment, Maillard reactions change the taste, flavour and colour of foods. The food industry is directly concerned with the occurrence of these reactions in processed foods. Food developers need to control recipes, processes and conditions of food preparation in order to optimise the organoleptic and nutritional

qualities of foods (Finot et al., 1990). Therefore, controlling the rate and the extent of such reactions is essential to obtain good quality products. Breakfast cereals contain reducing sugars derived from the starch present (see section 2.3.1.1), from the sugar syrups that usually contain glucose and from the sucrose if this sugar dissociates into glucose and fructose at temperatures above 70 °C (Davies and Labuza, 1997). Cereals also contain amine functions (from the proteins of cereal grains). The presence of both these compounds allows the Maillard reactions to occur at elevated temperatures (non enzymic browning). The production process of breakfast cereals involves many steps where Maillard reactions are favoured, thanks to the high temperatures applied to this intermediate moisture product, such as during the cooking and drying steps, but mainly during the toasting step.

There are three stages to the Maillard reactions: early, advanced and final. The first corresponds to the steps without browning, the second to the reactions leading to volatile or soluble substances and the third to the reactions generating insoluble brown polymers called melanoidins (Hodge, 1953).

- General mechanisms of the reactions

In the early stage of the Maillard reactions, a reducing sugar, like glucose, condenses with a compound possessing a free amino-group to give a Schiff base. The resulting Schiff base is labile and may undergo two sequential rearrangements yielding a reasonably stable aminoketose, the Amadori product. This Amadori rearrangement can take place spontaneously even at 25 °C (O'Brien et al., 1998). These reactions are presented in Figure 2.12 and Figure 2.13.

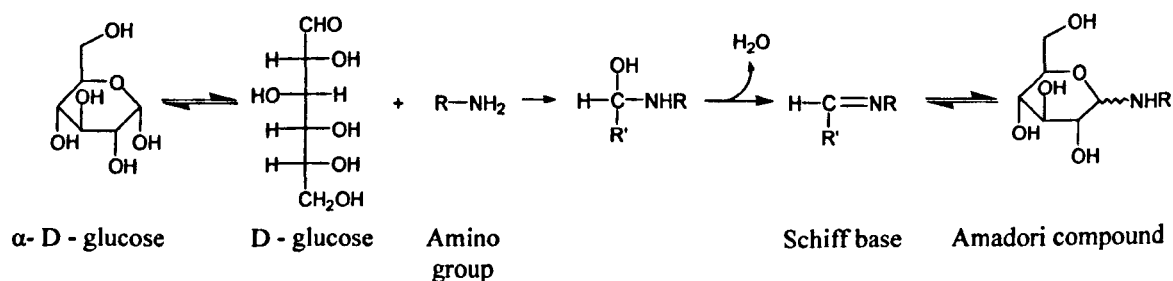


Figure 2.12: Initial step of the Maillard reactions with the formation of the Amadori compound.

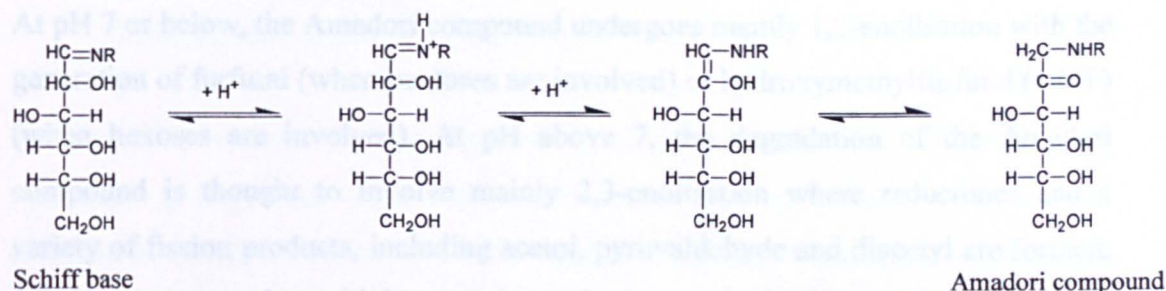


Figure 2.13: Amadori rearrangement.

The subsequent degradation of the Amadori product is dependent on the pH of the system, as presented in Figure 2.14. These reactions correspond to the advanced Maillard reactions.

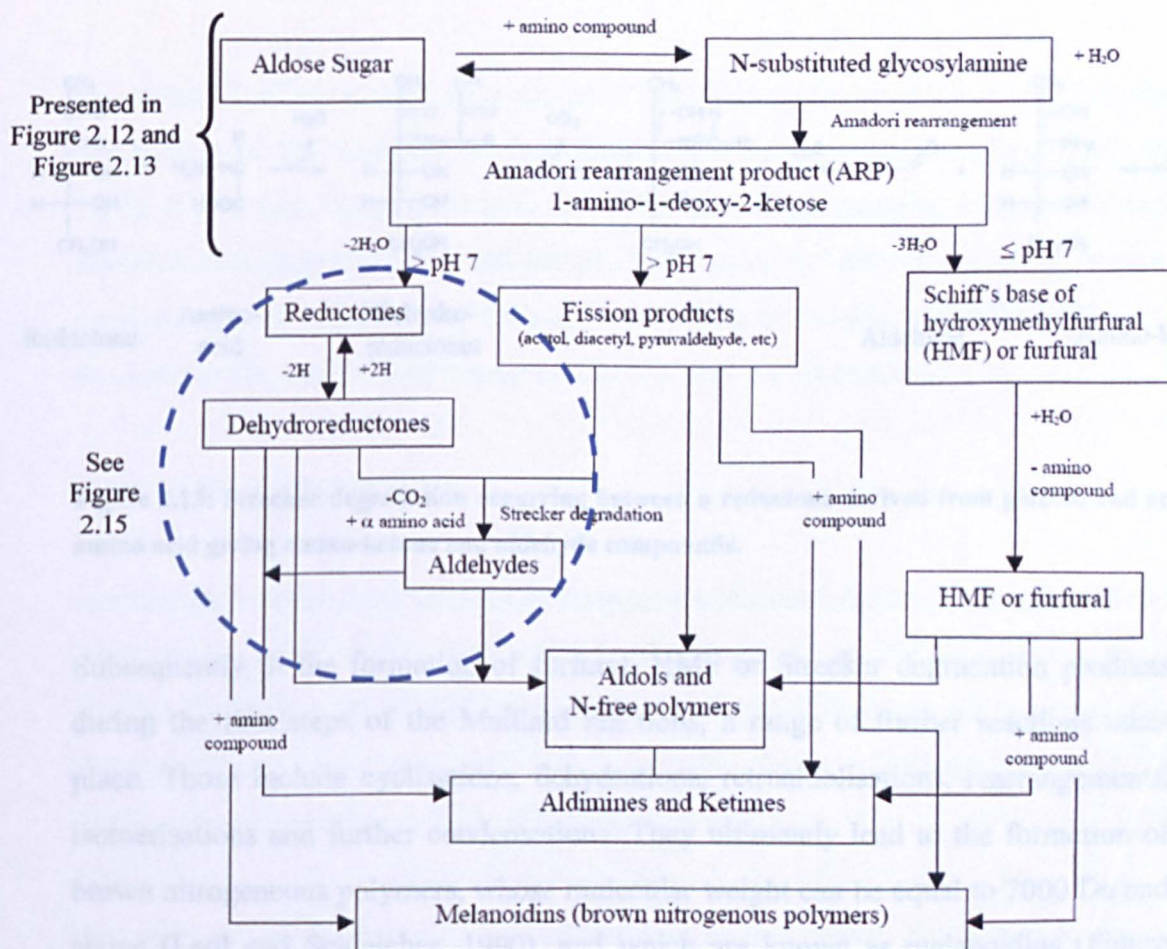


Figure 2.14: Maillard reactions scheme adapted from Hodge (1953).

At pH 7 or below, the Amadori compound undergoes mainly 1,2-enolisation with the generation of furfural (when pentoses are involved) or hydroxymethylfurfural (HMF) (when hexoses are involved). At pH above 7, the degradation of the Amadori compound is thought to involve mainly 2,3-enolisation where reductones and a variety of fission products, including acetol, pyruvaldehyde and diacetyl are formed. All these compounds are highly reactive and take part in further reactions (Mottram, 1994).

Carbonyl groups formed during the Amadori rearrangement can condense with free amino groups, which results in the incorporation of nitrogen into the reaction products. This reaction is known as the Strecker degradation. The Strecker degradation leads to the formation of CO₂, aldehydes and amino-ketones (Figure 2.15).

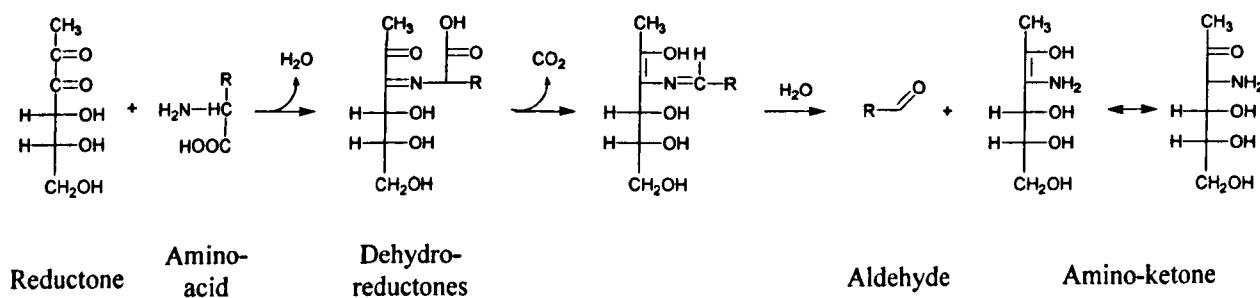


Figure 2.15: Strecker degradation occurring between a reductone derived from glucose and an amino acid giving amino-ketone and aldehyde compounds.

Subsequently to the formation of furfural, HMF or Strecker degradation products during the first steps of the Maillard reactions, a range of further reactions takes place. Those include cyclisations, dehydrations, retroaldolisations, rearrangements, isomerisations and further condensations. They ultimately lead to the formation of brown nitrogenous polymers, whose molecular weight can be equal to 7000 Da and above (Ledl and Schleicher, 1990), and which are known as melanoidins (Figure 2.14). These are the final Maillard reaction products generating colour.

- Consequences of Maillard reactions in food products

As presented above, Maillard reactions lead to the formation of hundreds of molecules giving foods their organoleptic qualities. Depending on the reaction conditions, the concentration of the intermediates, like the Amadori compounds, can vary over a significant range. As a consequence, the compositions of the degradation products differ and with that, the flavour and colour of the final product (Ledl and Schleicher, 1990).

Moreover, production of water occurs at several stages of the Maillard reactions (Figure 2.14). In low moisture foods such as breakfast cereals, this water formation might significantly increase the local moisture content, which could influence the rate of browning (Figure 2.16).

Lowering of pH is another consequence of Maillard reactions. pH greatly affects the mechanism of the Maillard reactions themselves, as previously explained (Figure 2.14). It may also cause polymeric material, particularly proteins, to lose solubility and thus alter their reactivity and the product texture (O'Brien et al., 1998). Hence, consequences of Maillard reactions on food products, such as water formation and decrease in pH, can have an impact on the chemical pathway taken.

- Acrylamide formation

Acrylamide is among the molecules formed via Maillard reactions (Mottram et al., 2002; Stadler et al., 2002). The presence of this compound in starchy based foods was highlighted in April 2002 by the National Food Administration in Sweden (Tareke et al., 2002). The molecule is a well known neurotoxin, and can be mutagenic and carcinogenic following long-term exposure.

Exposure can be high in certain occupational environments, but is low for most members of the public, the main exposure coming from packaging materials. It is estimated that the average UK consumer might consume 0.61 µg/kg of body weight per day from the diet, with this figure rising to 1.29 µg/kg of body weight for high adult-consumer exposure (Mill et al., 2008). There is, to date, no direct evidence that

any harmful effects have been caused by the presence of this molecule in food (Klaunig, 2008; Gerrard, 2006). However, due to the precaution principle, the acrylamide levels in food products should be monitored.

Acrylamide is mainly formed in heat-treated carbohydrate-rich foods (Taeymans et al., 2004). Asparagine, a major amino acid in potatoes and cereals, is a crucial participant in the production of acrylamide. Acrylamide can be formed during the Strecker degradation involving asparagine, in which 3-oxopropanamide and 3-aminopropanamide are key intermediates (Channell et al., 2008). Another mechanism leading to the formation of acrylamide in food is via the transformation of asparagine and a carbohydrate to a decarboxylated Schiff base. This Schiff base is then further transformed to acrylamide directly or via intermediate stages (Friedman and Levin, 2008).

The almost exclusive formation of acrylamide from asparagine could explain the occurrence of acrylamide in cooked plant based foods, such as cereals and potato, which are rich in this particular amino acid. For example, asparagine is present at 167 mg/kg in wheat flour, corresponding to 14 % of the total free amino acids (Mottram et al., 2002). Acrylamide was found to be significantly higher in wheat based breakfast cereals, followed by corn, oat and rice (Rufian-Henares et al., 2006). A study has shown that in the United States, breakfast cereal products are the fourth highest contributors to acrylamide intake leading to a mean acrylamide intake of 0.040 µg/kg of body weight per day with this type of food only (Friedman and Levin, 2008). The German federal office of consumer protection and food safety stated a signal value of 200 µg/kg for breakfast cereals. A signal value is defined as the lowest level of the 10 % food products containing the highest level of acrylamide. Acrylamide content in breakfast cereals depend on the product, hence the signal value varies depending on the market. When the signal value was measured in Spain, a level of 450 µg/kg was found (Rufian-Henares et al., 2006).

Some experiments revealed that in breakfast cereals, most or all of the acrylamide present is formed during the toasting step (Taeymans et al., 2004). Once acrylamide is formed, this compound is stable over prolonged storage periods of up to 12 months (Stadler, 2005).

- Factors affecting the Maillard reactions and possible impact of NaCl

In food products, the rate of Maillard reactions and the nature of the products formed are determined by the chemical composition (nature and concentration of the reactants) and the reaction conditions. These include time-temperature combinations, pH, water activity and water content. The physical state of the food product and the presence of ions and/or reaction inhibitors also have an influence (Van Boekel, 2001; Ellis, 1959).

An impact of NaCl on the chemistry of Maillard reactions was mentioned only in a limited number of publications. It was reported that the presence of NaCl in a reaction mixture of fructose and asparagine decreased the Schiff base formation, hence slowing the formation of some Maillard compounds such as acrylamide (Gokmen and Senyuva, 2007). Lindsay and Jang (2005) suggested that salts like CaCl_2 or FeCl_3 could be associated with the amino-acids via ionic interactions, minimizing the early-stage of Maillard reactions. It was also found that addition of cations such as Ca^{2+} and Mg^{2+} would change the reaction path from the Maillard reactions toward dehydration of glucose (Gokmen and Senyuva, 2007). Colour formation was inhibited by the presence of NaCl in glucose / amino-acid mixtures (Kwak and Lim, 2004) and caseinate / glucose mixtures (Pham and Cheftel, 1990). Hence, it seems that NaCl, as well as other salts, could influence directly the chemistry of Maillard reactions.

In breakfast cereal products, the NaCl level may also indirectly impact Maillard reactions by influencing the physical state of the product or the water evaporation during the toasting stage.

It was previously mentioned that, among other parameters, Maillard reactions can be influenced by the system's moisture content. Fernandez-Artigas et al. (1999) observed that Maillard reactions occurred slowly in dry food systems. It was also observed that samples of gluten and glucose heated under wet conditions produced more colour at all temperatures (80 °C, 100 °C, 120 °C, 150 °C) compared to those heated under dry conditions (Fogliano et al., 1999). It was suggested that the optimum reaction rate was for water activities (a_w) of 0.65-0.75 as shown in Figure

2.16 (Sensidoni et al., 1999; Mottram, 1994; Labuza et al., 1970). However, Sherwin and Labuza (2003) demonstrated that it is the mass of moisture, hence the moisture content, which should be correlated to the Maillard reactions rate rather than the a_w .

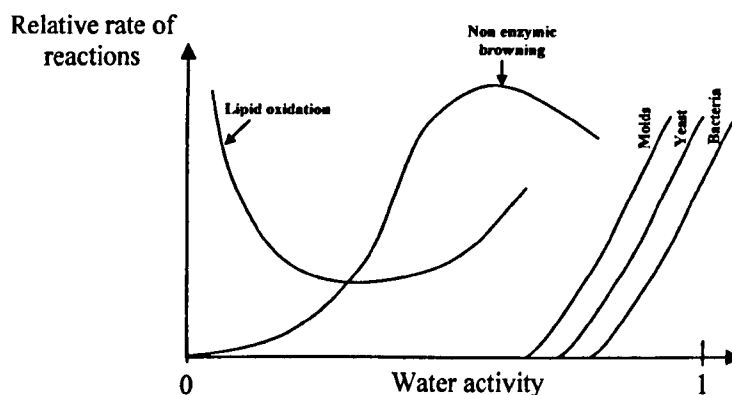


Figure 2.16: Dependence of food deterioration rates versus water activity (Labuza et al., 1970).

The decrease in rates below the maximum due to a lower moisture content is generally attributed to decreasing availability of water, which is necessary for mobility of the reactants (Sensidoni et al., 1999; Karel and Buera, 1993). Two approaches to describe this mobility are possible (Karel and Buera, 1993):

- In the “solution” scheme, it may be assumed that the Maillard reactions occur only in solution. At low moisture content values, no reactants are in solution, hence there is no reaction. Above this point, the concentration of reactants remains constant (because excess of solute maintains a saturated level), but the total volume in which the reaction takes place increases. The apparent reaction rate constant calculated for the total system increases because the reactive volume fraction increases. At the maximum reaction rate, all the reactants are in solution and further water additions result in dilution (Karel and Buera, 1993).
- In the “diffusion hypothesis”, the reduction in rate at low moisture content is attributed to diffusional limitations. Indeed, at low moisture contents, the diffusivity of sugars, various small organic molecules and water itself decreases

exponentially as water contents approach zero. Diffusion is a limiting factor for Maillard reactions as they consist at first in a bimolecular condensation step between glucose and amino-acids or proteins. Other condensation steps exist during the Maillard reactions, such as during the Strecker degradation, which could also be affected by the molecule diffusion (Karel and Buera, 1993).

NaCl is a hygroscopic compound, meaning that the presence of this salt in a system favours water sorption. It might be possible that this ability of sodium chloride to sorb water could also influence water evaporation from a system during a heat treatment. If NaCl could slow down water evaporation, Maillard reactions might be favoured as the reactants would be mobile longer thanks to the longer presence of water.

Maillard reaction rates are also influenced by the physical state of the system (glassy versus rubbery) as it changes the diffusion of the reactants. As mobility is much greater at temperatures above the glass transition temperature (Figure 2.11), it was observed that T_g has a significant influence on non-enzymic browning for both crystallizing and non-crystallizing systems, browning mainly occurring above T_g (Schebor et al., 1999; Karel and Buera, 1993). This hypothesis was observed in both model systems and vegetables. The Maillard reaction rate was very low in the proximity of the glass transition and increased exponentially as $T - T_g$ increased (Lievonon et al., 1998; Buera and Karel, 1995; Roos and Himberg, 1994; Karel and Buera, 1993; Karmas et al., 1992).

As sodium chloride has a low molecular weight, it might act as a plasticiser and decrease the glass transition temperature of breakfast cereal products. The presence of NaCl in the product might extend the time it is in a rubbery state when the flakes exit from the toasting tunnel, hence allowing Maillard reactions to proceed longer.

However, to a lower extent, browning was observed in glassy polymeric matrices of gelatinised starch, maltodextrin or poly(vinylpyrrolidone) kept well below T_g and in the virtual absence of water. Therefore, the T_g parameter should not be considered as an absolute threshold of stability with regard to non-enzymic browning reactions (Le Meste et al., 2002; Lievonon et al., 2002; Craig et al., 2001; Schebor et al., 1999;

Roos, 1995; Bell, 1995). Transport of water and other small molecules takes place at a significant rate even when the matrix is in the glassy state (Le Meste et al., 2002). Rotational mobility and diffusion through defects (cracks and pores) of the glasses may explain the occurrence of chemical reactions in the glassy state (Schebor et al., 1999).

Flavour, aroma and colour of breakfast cereals do not only come from Maillard reactions. Indeed, the use of high temperatures also favours caramelisation reactions, which are discussed in the next section.

2.4.2.2. *Caramelisation reactions*

Extensive literature can be found on the Maillard reaction and its mechanism. By contrast, only a limited number of publications focus on caramelisation in food products, although both mechanisms can happen at the same time during the heat treatment of a food product. Caramelisation results from a series of chemical reactions involving sugars, that include hydrolysis, dehydration, polymerisation, and lead to colour and volatile flavour and aroma compounds (Kitts et al., 2006).

Caramelisation occurs in food when these systems are heated above 70 °C (Davies and Labuza, 1997), for example during the toasting process of breakfast cereals. The flavours produced via caramelisation change from mild, caramel-like and sweet to burning bitter if the heat treatment is prolonged. Such reactions are favoured at pH values above 9 or under 3 (Kroh, 1994).

- General mechanisms of the reactions

The generation of flavour and colour in thermally induced caramelisation requires that sugars, normally monosaccharide structures, undergo first intermolecular rearrangements. Enolisation of the monosaccharides occurs (Figure 2.17), followed by a dehydration step (Figure 2.18) (Phongkanpai et al., 2006; Kroh, 1994).

Compounds such as 3-deoxyhexosulose formed via the reaction presented in Figure 2.18 are key intermediates of the caramelisation reactions. They lead to the formation of important volatiles characteristic of caramel flavour, giving a food product its specific taste. Typical components of caramel flavour are the furans (principally HMF), the furanones, the pyrones or the carbocyclics (Kroh, 1994; Pons et al., 1991). It should be noted that HMF is also a by-product of Maillard reactions. The polymerisation of molecules generated during caramelisation reactions is responsible for colour formation in a similar way as during Maillard reactions.

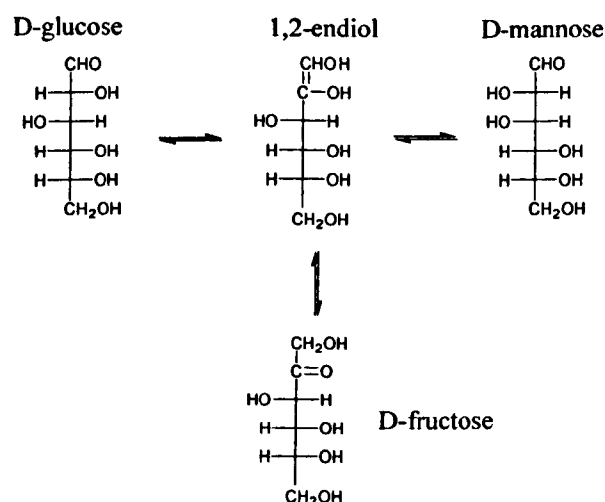


Figure 2.17: Monosaccharide enolisation.

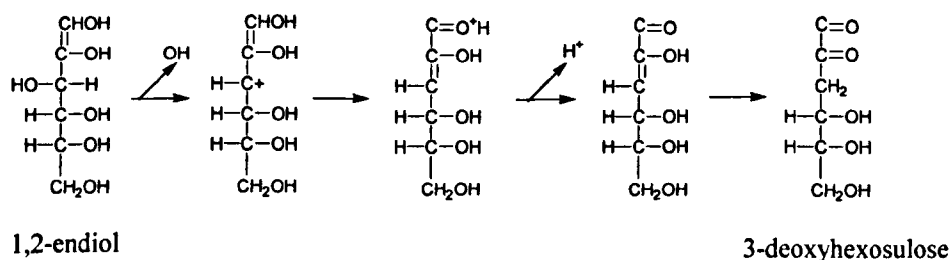


Figure 2.18: Dehydration step of the caramelisation reactions.

- Consequences of caramelisation reactions in food and impact of NaCl

Colour, aroma and flavour compounds are formed via caramelisation reactions changing the food's organoleptic characteristics. The relative proportion of each compound formed can be influenced by the temperature and the pH of the system (Kroh, 1994). As for Maillard reactions, the caramelisation reactions cause the

release of H^+ . Thus, the pH of the system undergoing caramelisation falls with time, enhancing the caramelisation reactions.

Some compounds are known catalysts for caramelisation reactions, such as ammonia and ammonium salts, acids, sulfites, hydroxides and basic amino-acids (Fadel and Farouk, 2002; Defaye and Ratsimba, 2000; Sikora and Krakow, 1994; Pons et al., 1991; Sikora and Tomasik, 1989). Impact of NaCl on caramelisation reactions was not found in the literature. However, considering the several catalysts of these reactions, NaCl could directly have an impact on the caramelisation reaction rate by influencing chemically the kinetics of these catalytic reactions.

Caramelisation reactions do not necessitate any bimolecular condensation at an early stage. Hence, the mobility of the molecules is not as critical here as it could be for Maillard reactions. Therefore, an indirect impact of NaCl on caramelisation by influence on the environment such as its physical state or moisture content is unlikely.

2.4.2.3. Influence of NaCl on flavour and aroma release

As previously presented, Maillard and caramelisation reactions lead to the formation of flavour or aroma molecules during the heating step. These molecules can have a relatively small molecular weight and can be volatile. Some of them can be released during the heating step, and others are the residual volatile molecules remaining entrapped in the products. They might be then released continuously during product storage and/or during the consumption of the product when the cereals are mixed with milk or the consumer's saliva. Mixing the product with an aqueous media such as milk or saliva helps the release of flavour molecules. Indeed, flavour molecules are generally hydrophobic and poorly soluble in water (Covarrubias-Cervantes et al., 2004). Hence, their release is favoured by hydration of the product. At that stage, they might contribute to the overall organoleptic characteristics of the products.

As previously explained, NaCl might influence Maillard and caramelisation reactions, either directly or indirectly, modifying the nature and amount of volatile molecules formed. The presence of NaCl can also affect the rate these volatiles are

released from the product. Water soluble molecules such as NaCl can bind a considerable amount of water to build hydration shells during solubilisation. If flavour molecules are also present in the solution, their release is increased due to the decreased availability of water molecules for the solubilisation of flavour compounds. Indeed, the affinity of water for the ions is considerably stronger than for the volatiles. Consequently, this leads to a decrease of free water volume and an increase of initial flavour concentration in the remaining free water. This increase of the volatile release due to the presence of salts such as NaCl is called the salting-out effect (as explained in section 2.3.2.2 for NaCl and proteins) (Rabe et al., 2003). This salting-out effect can have an impact on the product aroma. Indeed, it was previously observed that adding NaCl to an aqueous system increased its aroma intensity and the volatile headspace concentration for NaCl concentrations of 2 % (wwb) (Ebeler et al., 1988; Poll and Flink, 1984).

During the toasting stage, the product acquires its main physical and organoleptic characteristics as this product undergoes a state transition (rubbery to glassy) and Maillard and caramelisation reactions generate flavour and colour. Another product modification should also be considered during this process step: the use of high temperatures in a dry environment can alter the starch molecules, as presented below.

2.4.3. Starch degradation

During the toasting stage, the breakfast cereal flakes' temperature reaches 180 °C or above, as the flakes are almost dry and the surrounding air is between 180 and 235 °C, depending on the process used. At such temperatures, the starch may start to depolymerise leading to the formation of smaller molecules such as glucose (Aggarwal and Dollimore, 1996; Fujio et al., 1995; Tomasik et al., 1989; Davidson et al., 1984). Pyroconversion is the dry roasting of acidified starch, during which their α 1-4 bonds are hydrolysed and shorter soluble glucose chains are formed (dextrins) (Van Beynum and Roels, 1985). A schematic of the phenomenon is presented in Figure 2.19. The toasting temperatures vary between 110 °C and 200 °C depending on the type of products wanted (Thomas, 1999).

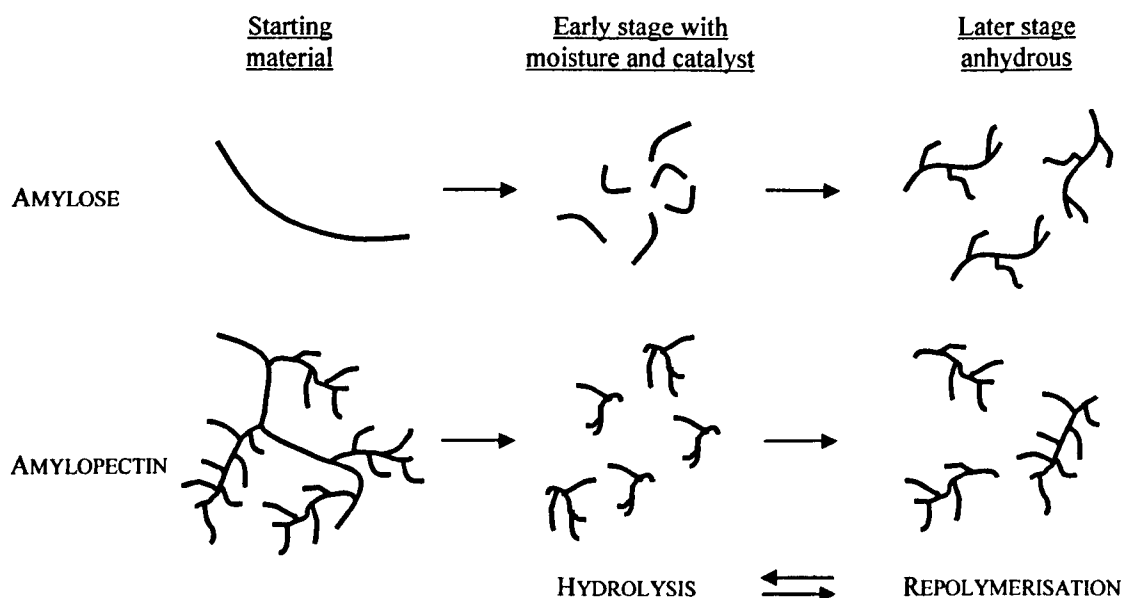


Figure 2.19: Molecular transformations that occur during pyroconversion (Thomas, 1999).

Depending on the reaction conditions (e.g. pH, moisture, temperature and heating time), pyroconversion produces a range of products that vary in viscosity, cold-water solubility, colour, reducing sugar content and stability. It was reported that the solubility of native starches increases with the heating time for temperatures of 150 to 175 °C due to the dextrinisation of the molecules. At temperatures between 200 and 213 °C, maximum solubility is reached. Above this temperature, a decreased solubility was interpreted as the results of secondary reactions of retrogradation or polymerisation of the oligosaccharides formed in the first dextrinisation step (Tomasik et al., 1989). Indeed, depending on the conditions, both hydrolysis and repolymerisation can occur (Figure 2.19) (Thomas, 1999). At the end of the pyroconversion process, the moisture content of the products ranges between 0.5 and 3 % moisture. Acid catalysts favour the hydrolysis of the starch glucosidic bonds. In native starches, amorphous parts of the granules are hydrolysed before the crystalline parts (Hoover, 2000). Presence of amino-acids with starch during a dry heat treatment also depresses the decomposition point of starch (Kapusniak et al., 1999).

Starch polymer hydrolysis, catalysed by heat, could occur in breakfast cereal products during toasting. Indeed, this starch based product is heated in a dry environment for temperatures generally exceeding 200 °C and the final moisture

content of breakfast cereal is similar to those of the pyroconverted products. Moreover, Maillard and caramelisation reactions are occurring, leading to the formation of H^+ . The pH of the system is therefore lowered, favouring starch hydrolysis. The glucose that could be formed might then caramelize or be involved in further Maillard reactions.

Presence of NaCl in breakfast cereal products could have an impact on the possible starch hydrolysis occurring during the toasting step. This impact could be direct, such as follows: NaCl is a non-transition metallic salt known for its efficient heat conduction. It was reported that adding NaCl to a starch mixture before its extrusion led to the formation of products with a decreased expansion. It was suggested that the presence of NaCl increased the heat conduction of the system, which might have caused an enhanced starch molecular breakdown explaining the expansion decrease (Chinnaswamy and Hanna, 1988). Bryce and Greenwood (1963) reported that presence of NaCl with amylo maize starch heated between 220 and 340 °C in high vacuum lowered the threshold of pyrolysis temperature and led to the formation of anhydro-glucose. Catalytic effects of several salts on amylose decomposition were also observed by Desai et al. (1972). Acid hydrolysis of starch was reported to be enhanced by the presence of NaCl and other salts, yielding higher glucose formation (Kunlan et al., 2001; Van Beynum and Roels, 1985). Based on these findings, one could assume that the presence of NaCl might enhance directly a possible starch hydrolysis catalysed by heat during the toasting step of breakfast cereal products.

Presence of NaCl could also have an indirect impact on a possible starch hydrolysis. If this salt affects the rate and/or the pathways of Maillard and caramelisation reactions, the amount of H^+ released in the food system might vary. As acidic products are known catalysts for starch pyroconversion, the level of NaCl in breakfast cereal products might indirectly affect their amount via an influence on the Maillard and caramelisation reactions.

Based on this literature review concerning breakfast cereals' manufacturing process and ingredients, and the possible impacts of NaCl, an in-depth study of the influence of NaCl on the quality of breakfast cereals was carried out. The main aims and objectives of this study are presented in the following section.

2.5. Aims and objectives of the study

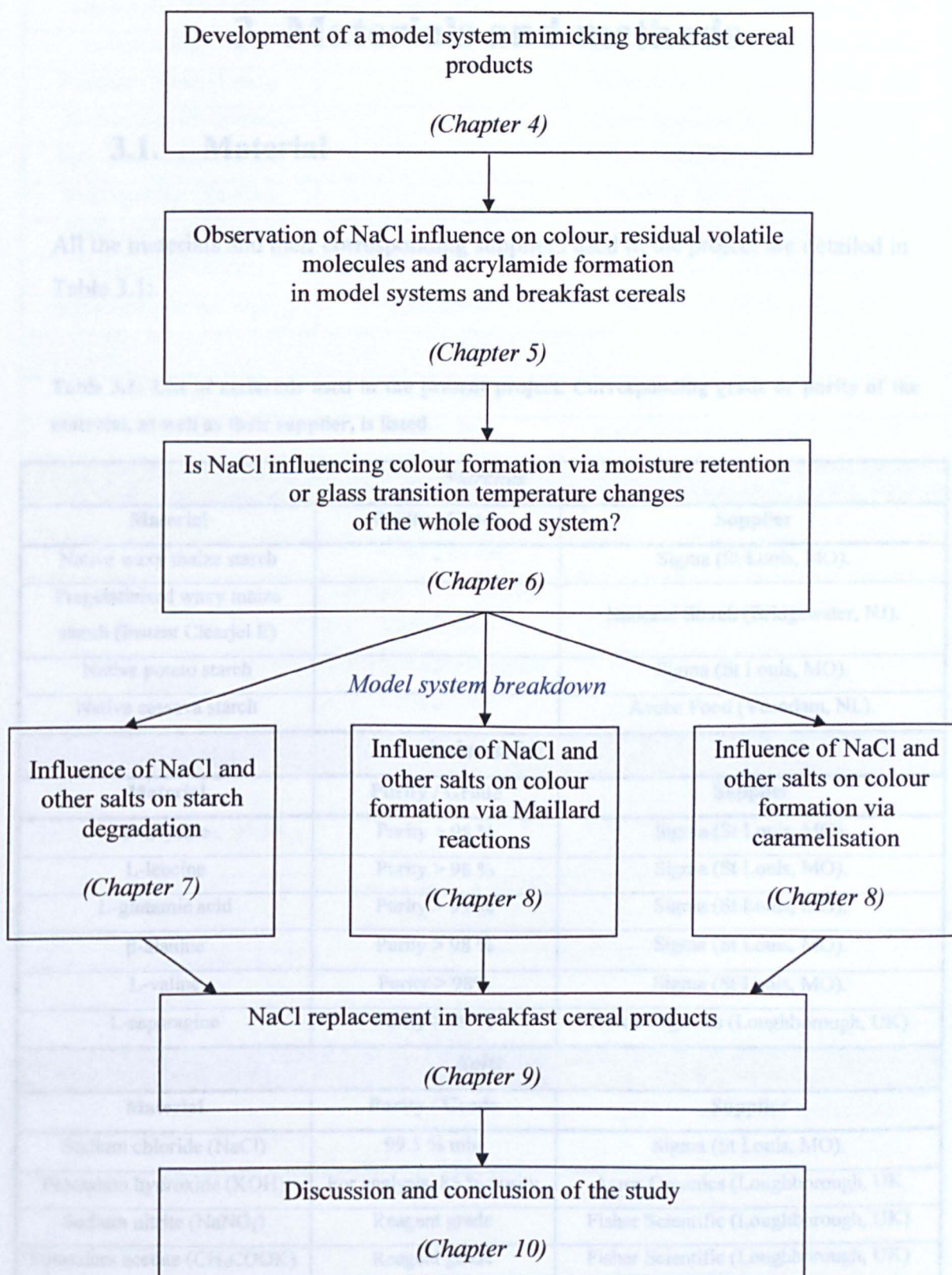
In order to decrease the level of NaCl in breakfast cereal products without altering the product quality (taste, colour, aroma, texture...), it is necessary to understand the role of NaCl in this type of food. Hence, the main objectives of the project described in this thesis were:

- (1) To observe the impact of NaCl on several quality parameters of breakfast cereal products.
- (2) To investigate possible ways by which NaCl is having such an influence on breakfast cereal products.
- (3) To suggest alternative ways to get the same impact as sodium chloride on the breakfast cereal products, but using other ingredients and/or processes.
- (4) To verify whether these alternative ways to compensate a sodium chloride reduction were efficient. This was done by implementing them during breakfast cereal production and assessing the resulting product quality.

At first, a model system for ready-to-eat breakfast cereals was developed and was used to follow the NaCl influence on colour, some residual volatile molecule release and acrylamide formation. The results were then transferred to breakfast cereal products to confirm the results obtained with the model systems. The second part of the present study aimed to investigate two possible ways NaCl may influence colour formation via its potential impact on molecular mobility. In a third part, the model system was broken down in order to understand the impact of sodium chloride more specifically on each component of the system. The NaCl influence on colour formation via starch degradation during a heat treatment, via Maillard reactions and caramelisation were investigated. Finally, the last chapter reports the transfer of the findings to breakfast cereal products by the testing of new product formulations.

A schematic representation of the thesis organisation, chapter by chapter, is presented in the next section.

2.6. Thesis organisation



3. Materials and methods

3.1. Material

All the materials and their corresponding suppliers used in the project are detailed in Table 3.1:

Table 3.1: List of materials used in the present project. Corresponding grade or purity of the material, as well as their supplier, is listed.

Starches		
Material	Purity / Grade	Supplier
Native waxy maize starch	-	Sigma (St Louis, MO).
Pregelatinised waxy maize starch (Instant Clearjel E)	-	National Starch (Bridgewater, NJ).
Native potato starch	-	Sigma (St Louis, MO).
Native cassava starch	-	Avebe Food (Veendam, NL).
Amino-acids		
Material	Purity / Grade	Supplier
L-Glycine	Purity > 98 %	Sigma (St Louis, MO).
L-leucine	Purity > 98 %	Sigma (St Louis, MO).
L-glutamic acid	Purity > 99 %	Sigma (St Louis, MO).
β-alanine	Purity > 98 %	Sigma (St Louis, MO).
L-valine	Purity > 98%	Sigma (St Louis, MO).
L-asparagine	Purity > 99 %	Acros Organics (Loughborough, UK)
Salts		
Material	Purity / Grade	Supplier
Sodium chloride (NaCl)	99.5 % min	Sigma (St Louis, MO).
Potassium hydroxide (KOH)	For analysis, 85 % purity	Acros Organics (Loughborough, UK)
Sodium nitrite (NaNO ₂)	Reagent grade	Fisher Scientific (Loughborough, UK)
Potassium acetate (CH ₃ COOK)	Reagent grade	Fisher Scientific (Loughborough, UK)
Potassium carbonate (K ₂ CO ₃)	Reagent grade	Fisher Scientific (Loughborough, UK)
Diphosphorus pentoxide (P ₂ O ₅)	Reagent grade	Fisher Scientific (Loughborough, UK)
Potassium chloride (KCl),		Sigma (St Louis, MO).

Calcium chloride (CaCl ₂),		Sigma (St Louis, MO).
Sodium carbonate (Na ₂ CO ₃)	99 % min	Sigma (St Louis, MO).
Lithium chloride (LiCl)	99 % min	Fisher Scientific (Loughborough, UK)
Sodium iodide (NaI),		Fisher Scientific (Loughborough, UK)
Potassium nitrate (KNO ₃),		Fisher Scientific (Loughborough, UK)
Sodium bromide (NaBr),		Fisher Scientific (Loughborough, UK)
Potassium bromide (KBr),		Fisher Scientific (Loughborough, UK)
Sodium sulfate (Na ₂ SO ₄),		Fisher Scientific (Loughborough, UK)
Sodium nitrate (NaNO ₃)		Fisher Scientific (Loughborough, UK)
Potassium iodide (KI)		Fisher Scientific (Loughborough, UK)
Magnesium chloride (MgCl ₂)	Anhydrous	DH Scientific (Huddersfield, UK).
Sugars		
Material	Purity / Grade	Supplier
D-glucose	Purity > 99 %	Fisher Scientific (Loughborough, UK)
Trehalose	Purity > 99 %	Acros Organics (Loughborough, UK)
Material for acrylamide analysis		
Material	Purity / Grade	Supplier
Water	For liquid chromatograph LiChrosolv®	Merck (Darmstadt, DE)
Methanol	For liquid chromatograph LiChrosolv®	Merck (Darmstadt, DE)
[¹³ C ₃]-Acrylamide	98 %,	Cambridge Isotope Laboratories Inc., (Cambridge, UK)
Dichloromethane (min. 99.9 %),	For liquid chromatograph LiChrosolv®	Merck (Darmstadt, DE).
Formic acid	Electrophoresis grade 98- 100 % for analysis	Merck (Darmstadt, DE)
Potassium hexacyanoferrate (II) trihydrate	For analysis	Merck (Darmstadt, DE)
Sodium chloride	For analysis	Merck (Darmstadt, DE)
Zinc sulfate heptahydrate	For analysis	Merck (Darmstadt, DE)
Isolute multimode cartridges		International Sorbent Technology (Tucson, AZ)
Potassium hexacyanoferrate (II)- 3-hydrate, solution at 150 g/l		Fisher Scientific (Loughborough, UK)
Zinc sulfate-7-hydrate, solution at 300 g/l		Fisher Scientific (Loughborough, UK)
Ethyl acetate	For liquid chromatograph LiChrosolv®	Merck (Darmstadt, DE)

3.2. Methods

3.2.1 Sample preparation

Several types of model systems and breakfast cereals were used throughout this study. Although their compositions varied depending on the topic, the procedures to prepare the samples were the same. Therefore, the following sections focus on the mode of preparation of the samples depending on their types. The compositions of the model systems are presented at the beginning of each chapter for clarity.

3.2.1.1. *Model systems containing native starch*

Every component of the model systems (native starch, water and possible other minor components for a total of 100 g wet weight) were mixed in a container to obtain a visually homogeneous suspension. If the pH of the suspension needed to be adjusted, it was done at that stage. The pH was adjusted by adding KOH (5 M solution) to the suspension while this suspension was stirred with a magnetic bar to ensure pH homogeneity. The pH was measured with a pH meter.

The model system suspensions were dried overnight in a convection oven at 55 °C (MOV-112F, Sanyo Electric-Biomedical Co, Ltd, Wood Dale, IL). The samples were then weighed and water was added to the systems in order to reach a moisture content of 20 % \pm 1% (wwb). After milling the hydrated samples into a fine powder (Knifeter 1095 Sample Mill from Foss Tecator, Hoganas, Sweden), the moisture content was checked by a gravimetric method (as explained in section 3.2.2.1). The models were then sealed in aluminium bags until the heat treatment. Aliquots of hydrated samples (the weights of the aliquots used are mentioned in each chapter) were placed in open containers and heated in an oven (Thomas Collins & Co Ltd., Bristol, UK) for different periods of time and temperatures. The specific conditions used for a study are mentioned at the beginning of the corresponding chapter. The samples were then removed from the oven and left at room temperature for cooling and were milled into powders with a blender (Braun 4041-621, Barton upon Humber, UK) for further analysis.

3.2.1.2. Model systems containing pregelatinised starch

The model system components (pregelatinised starch, water and possible other minor components) were mixed together using a blender in order to obtain a clear paste. The pastes were frozen overnight at -20 °C and freeze dried (SuperModulyo Pirani 1001, Thermo Fisher Scientific Inc., Waltham, MA). After milling the samples into a fine powder, the moisture content was measured and the models were re-hydrated by storing the powders at a relative humidity (RH) of 100 % until the moisture content reached 15 % (± 1 % ww). The mixture was regularly manually stirred during the re-hydration step. The moisture content was checked (procedure detailed in section 3.2.2.1) and the models were sealed in aluminium bags until the heat treatment. Aliquots (the weights of the aliquots used are mentioned in each chapter) were placed in open containers and were heated in an oven. After the heat treatment, the samples were left to cool down at room temperature and were milled into powders with a blender for further analysis.

3.2.1.3. Model systems containing only glucose and salts

The model system components (glucose, salts and water) were weighed and poured into a plastic tube fitted with a screw cap to ensure that the tube was hermetically sealed. The mixture was heated at 95 °C in a water bath until complete dissolution of the glucose and salts. The weight of the container was measured before and after heating to ensure that no water evaporated during this heat treatment. Ten grams of the solution was then poured in an open aluminium container and heated in an oven for different times and temperatures. Once the samples were removed from the oven and cooled down to room temperature, 10 g of distilled water was added in each sample to dissolve it. The analyses were performed on these solutions.

3.2.1.4. Model systems containing only glucose, amino-acids and salts

A primary solution was prepared: the following components (Table 3.2) were weighed and poured into an Erlenmeyer flask.

Table 3.2: Composition of the primary solution used to prepare the model systems containing glucose, amino-acids and salts.

Material	Weight (g)
Leucine	5.880
Asparagine	3.000
Valine	2.112
Alanine	1.272
Glutamic acid	20.520
Glucose	43.200
Water	720.000

The vial containing the glucose, amino-acids and water was hermetically closed and heated at 95 °C in a water bath until complete dissolution of all components. The vial was weighed before and after the heat treatment; some water was then added to compensate for any weight loss due to water evaporation. Aliquots of this solution (33.160 g) were weighed and poured into open individual containers. Hence, each of them contained the same amount of glucose and amino-acids. Salts were then added to the solutions to obtain different concentrations. The type of salt and the quantity added to each sample depended on the experiments. The experimental conditions used are mentioned at the beginning of the corresponding chapter. After stirring the samples to ensure that the salts were dissolved, the samples were frozen at -20 °C and freeze dried. Water was then added to each sample to reach a specific moisture content, which was calculated based on the weight of dry matter present in each sample. As for the salt addition, the amount of water added to reach a certain moisture content depended on the experimental design, and is mentioned at the beginning of the corresponding chapter. After stirring the samples manually, they were heated in an oven for different times and temperatures. Following this heat treatment, 50 g of water was added to dissolve the samples. Some analyses were then performed on these solutions.

3.2.1.5. Breakfast cereal products' preparation

Several breakfast cereal recipes were made at the beginning of the project with the facilities in the pilot plant of Cereal Partners Worldwide (Welwyn Garden City, UK). Samples of wheat, wheat and rice mixture, corn and rice breakfast cereals containing different levels of NaCl were produced. The process used followed that presented in

section 2.3 of the literature review and is briefly summarized in Figure 3.1. Several aliquots of each recipe products were sampled throughout the process. For clarity purposes, these samples were named according to the processing step from which they were taken (Figure 3.1):

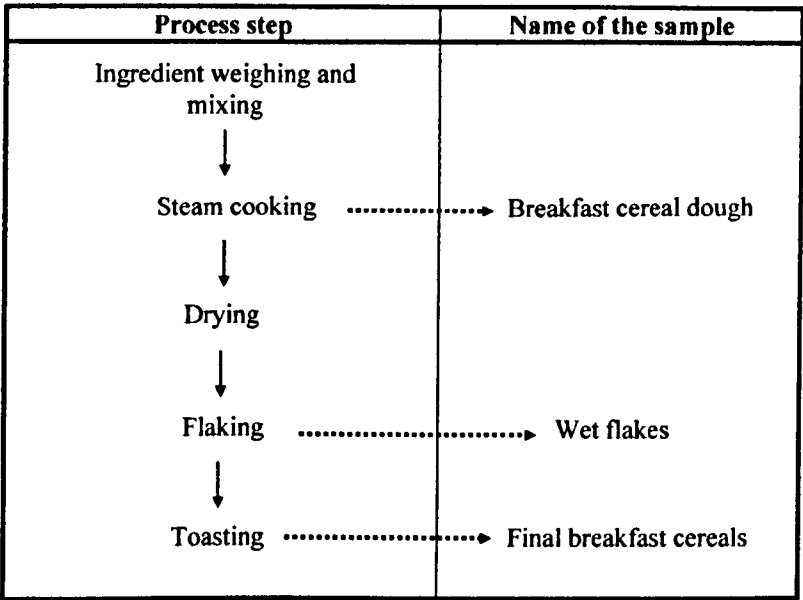


Figure 3.1: Process used for breakfast cereal production.

Different levels of NaCl were tested in wheat, wheat and rice mixture, corn and rice breakfast cereals (the concentrations were chosen based on commercial recipes). For each of them, a control sample without NaCl was prepared. For the wheat breakfast cereals, an intermediate NaCl concentration was also made. Table 3.3 summarizes the concentrations of NaCl used in each recipe for the different types of breakfast cereals produced.

In total, 9 types of breakfast cereal dough and wet flake samples were made, differing on the botanical origin of the cereal grains and on the NaCl level. Several toasting temperatures were used for the production of the final breakfast cereals. Each of the 9 breakfast cereal recipes was toasted at 2 or 3 different temperatures, depending on the type of breakfast cereals (Table 3.4). Hence, 23 samples of final breakfast cereals were produced.

Table 3.3: Levels of NaCl for the nine breakfast cereal samples produced.

Breakfast cereal type	NaCl concentrations used (% dwb)		
Wheat	0.00	0.75	2.50
Mixture of wheat and rice	0.00	-	1.32
Corn	0.00	-	2.33
Rice	0.00	-	1.68

Table 3.4: Toasting temperatures used depending on the breakfast cereal type.

Breakfast cereal type	Toasting temperatures used (°C)		
Wheat	180	205	230
Mixture of wheat and rice	180	190	200
Corn	230	240	-
Rice	200	215	-

Wheat and rice mixture, corn and rice breakfast cereals were toasted entirely in the pilot plant with the toasting tunnel using the temperatures described in Table 3.4.

Wet wheat breakfast cereal flakes were toasted using a lab-scale toasting device (Jetzone driers ovens 9247, Wolverine Massachusetts Corp., Merrimas, MA) at the temperatures presented in Table 3.4. The utilisation of this small toasting device allowed more flexibility for the toasting times and temperatures compared to the toasting tunnel used for the production of the final breakfast cereals. The use of this small toasting device ensured that the toasting was equivalent for each breakfast cereal flake, as it was equipped with a ventilation system mimicking the toasting tunnel used for the final breakfast cereals (Figure 3.2). The following settings were applied: supply fan speed adjusted to 7 and exhaust damper position adjusted to 2. The fan speed was set for the flakes to float at about 10 cm off the bottom of the rotative bowl to ensure homogeneous heat diffusion among the flakes. The exhaust damper position corresponded to the amount of air recycled to heat up the flakes. Hence, it controlled the humidity loss of the flakes during the toasting step.

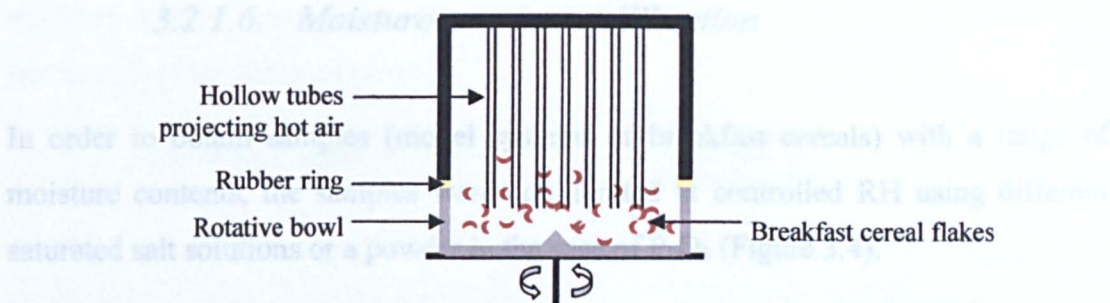


Figure 3.2: Representation of the lab-scale toasting device while toasting wet breakfast cereal flakes.

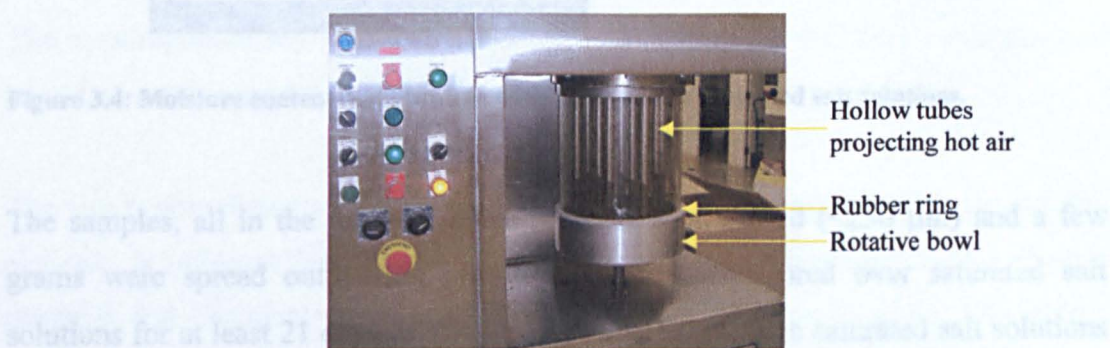


Figure 3.3: Picture of the lab-scale toasting device.

Breakfast cereal doughs were toasted using the oven that was previously used for the heat treatment of the model systems. These doughs were intermediate samples between the model systems that were entirely made in the lab and the breakfast cereals (wet flakes or final breakfast cereals) that were entirely made with larger scale food production equipments.

In summary, several types of breakfast cereal samples were made:

- breakfast cereal doughs (9 samples with different cereal sources and NaCl levels, toasted in the oven used for the model systems)
- wet flakes (9 samples with different cereal sources and NaCl levels, the wheat flakes were toasted with the lab-scale toasting device for different times and temperatures)
- final breakfast cereals (23 samples with different cereal sources, NaCl levels, and toasting temperatures, toasted in the toasting tunnel)

3.2.1.6. Moisture content equilibration

In order to obtain samples (model systems or breakfast cereals) with a range of moisture contents, the samples were equilibrated at controlled RH using different saturated salt solutions or a powder in the case of P_2O_5 (Figure 3.4).

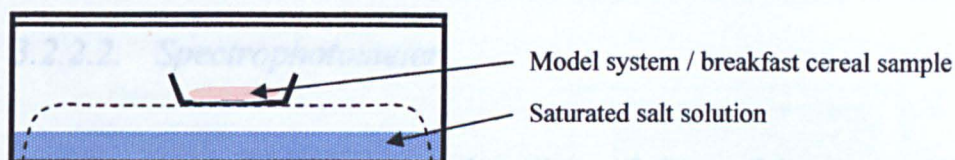


Figure 3.4: Moisture content equilibration of samples above saturated salt solutions.

The samples, all in the form of a fine powder, were sieved ($<250\ \mu\text{m}$) and a few grams were spread out within plastic containers and stored over saturated salt solutions for at least 21 days in hermetic boxes at $25\ ^\circ\text{C}$. The saturated salt solutions used to achieve various relative humidities were made using LiCl, CH_3COOK , K_2CO_3 , NaNO_2 , NaCl and water which gave water activity values of 0.11, 0.23, 0.43, 0.64, 0.75 and 1.00 respectively (Biliaderis et al., 1999). The P_2O_5 powder was used to obtain a relative humidity of 0 %. The moisture content of a sample was measured before and after storage (method presented in section 3.2.2.1) to ensure that all the samples were in the sorption phase during moisture equilibration.

3.2.2. Analytical techniques

Several techniques were used to characterise the model systems and the breakfast cereal products. Each of them is presented in this section.

3.2.2.1. Moisture content determination

The moisture content of a sample was determined using an oven drying method. The samples were dried by storage in an oven (Convection Oven MOV-112F, Sanyo Electric-Biomedical Co, Ltd, Wood Dale, IL) at $105\ ^\circ\text{C}$ for 18 h. A sample's moisture content was determined using the average of three measurements. All the

moisture content values were calculated as shown in Equation 3.1 and reported as a percentage of the sample (wwb):

$$\text{Moisture content} = \left(\frac{\text{Weight of water evaporated}}{\text{Weight of the sample before drying}} \right) \times 100$$

Equation 3.1

3.2.2.2. Spectrophotometer

The spectrophotometer was used to assess the colour of the model systems and breakfast cereal samples.

The eye is able to perceive colour when an incident light, coming from the sun for example, is reflected from an object to the eye in a way determined by the object's physical and chemical properties. The presence of chromophores on this object causes the specific absorption or scattering of certain wavelengths of light, leaving the remainder of the incident light to travel onwards to the observer (Macdougall, 2002). The human eye then detects the reflected wavelengths of the visible light (between 380 and 700 nm) and the brain interprets the message as a colour. To detect the wavelengths, the eye possesses two types of receptor: the cones and the rods, but only the cones are sensitive to colour. There are three types of cones, ρ , γ and β , each sensitive to a specific wavelength range corresponding to the primary lights, red, green and blue (Macdougall, 2002).

The CIE (Comité International d'Eclairage) system of colour measurement transforms the reflection or transmission spectrum of an object into a three dimensional colour space. This 3 dimensional colour space was based on how the eye is working. Therefore, the X axis represents the red colour, Y the green and Z the blue. These primaries do not exist as real lights, but they encompass all colours. Other colour scale systems were derived from this XYZ colour space. Among them, the $L^*a^*b^*$ colour space provides the values of three colour components: L^* (black-white component, luminosity) and the two chromatic coordinates, a^* (+ red to - green component) and b^* (+ yellow to - blue component). Calculations allow switching from one system to another.

- Principle

A spectrophotometer works by measuring the whole spectrum of visible light reflected from a sample. Measurements are taken via a diffraction grating of the reflected light from a sample, as represented in Figure 3.5. The results are expressed as the ratio between this light and the one reflected from a known reference standard (such as the light trap and the white tile).

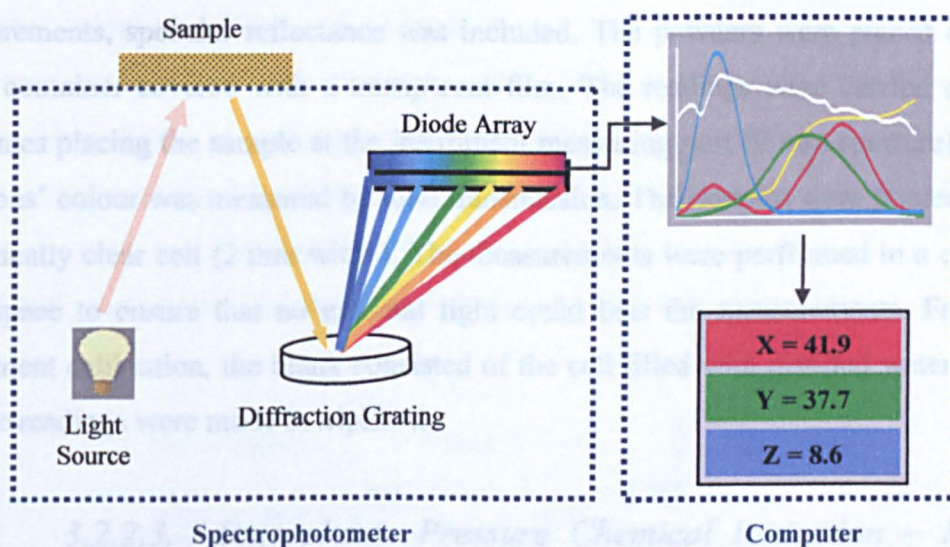


Figure 3.5: Representation of a colour measurement by reflectance using a spectrophotometer (Hunter Lab Corporation, 2001).

It is also possible to measure colour by transmission instead of reflectance in the case of non opaque samples, for example for some liquid samples. The same principle as the one explained above apply, except that the light measured by the spectrophotometer is not that reflected by the sample, but the one that travelled through it (Figure 3.6).

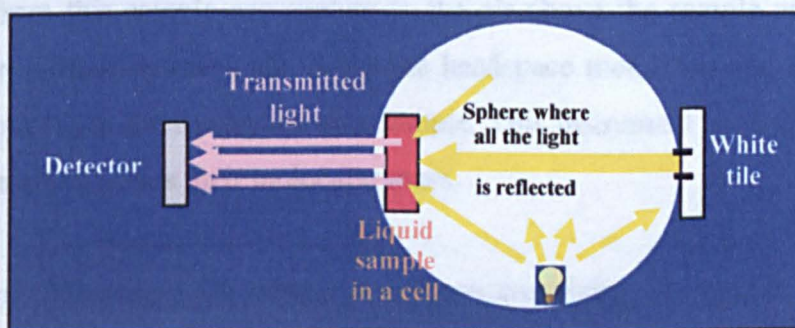


Figure 3.6: Representation of a colour measurement by total transmission using a spectrophotometer (Hunter Lab Corporation, 2001).

- Experimental procedure

The colour evaluations of the model systems were carried out using a tristimulus spectrophotometer (ColorQuest XE, Hunter Associates Laboratory, Reston, VA) operating in the CIE L*a*b* colour space. The instrument was calibrated with a white calibration tile and a light trap. The following measurement conditions were applied: standard illuminant D65 and observer angle 10 °. For the powder colour measurements, specular reflectance was included. The powders were placed into a small container covered with a transparent film. The readings were carried out in triplicates placing the sample at the instrument measuring port (9 mm aperture). The solutions' colour was measured by total transmission. The samples were poured into an optically clear cell (2 mm width). The measurements were performed in a closed dark space to ensure that no external light could bias the measurements. For the instrument calibration, the blank consisted of the cell filled with distilled water. The sample readings were made in triplicate.

3.2.2.3. Atmospheric Pressure Chemical Ionisation – Mass Spectrometry (APCI-MS)

The APCI-MS was used to quantify low-mass, volatile organic compounds released from model systems and breakfast cereal products.

- Principle

By storing a sample for a certain length of time in a closed container, the volatiles released from this sample accumulate in the air above the sample until it reaches equilibrium. These volatiles are called the headspace molecules. An APCI-MS can be used to analyse the headspace of a sample. This instrument produces a spectrum of ion intensities versus their molecular mass.

During a measurement, the sample headspace containing the volatile molecules is drawn through a silica capillary. Heat is applied at the end of the capillary ($T = 200\text{ }^{\circ}\text{C}$) to avoid condensation and assist desolvation of the volatiles. The molecules

then reach a high voltage electrode; the corona discharge (4 kV) from this electrode transforms the volatiles into ions by the addition of a proton to each molecule. Once the ions are formed, they are transferred to a mass spectrometer (MS) and analysed by a mass filter.

The mass spectrometer separates the ions according to their individual mass-to-charge ratio (m/z) using electric and/or magnetic fields. The charge of all the molecules being +1 (addition of a proton during the ionisation), they are therefore separated based on their molecular mass only. The mass detected by the MS is the mass of the molecule +1 (mass of a proton) (Figure 3.7) and designated as MH^+ . The mass spectrometer can be operated in two modes. Full scan mode is where all ions within a range are detected; SIM mode (selected ion monitoring) is where preselected ion masses are detected allowing enhanced sensitivity.

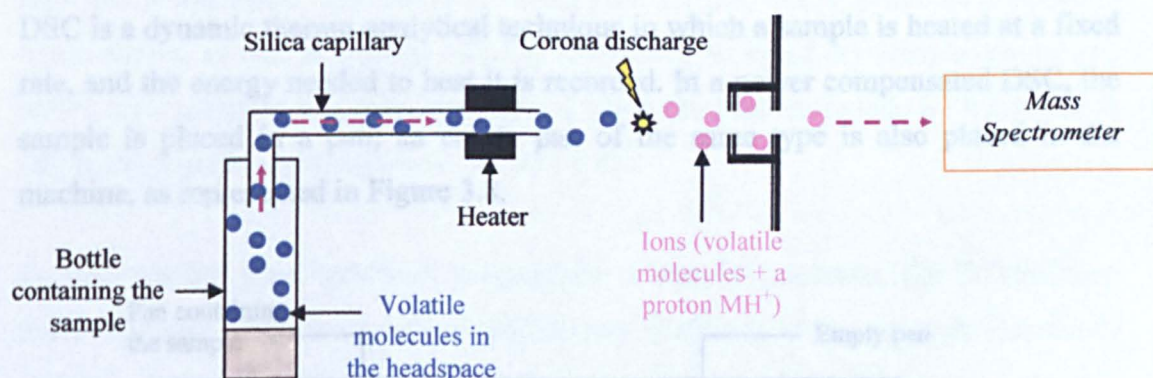


Figure 3.7: Representation of the reactions happening during the measurement of volatile molecules using an APCI-MS.

- Experimental procedure

Model systems or breakfast cereal powders (1.000 g) were transferred into a 28 ml bottle containing distilled water (2.000 g). The bottles were closed with a one-port lid, hand-shaken and left at ambient temperature (22 °C) for at least 16 h to allow volatile compound equilibration in the headspace. The headspace was sampled through the APCI-MS (Fisons Instruments, Beverly, MA) with an air flow of 10 ml/min. For SIM or full scan analyses, cone voltages of 15 V and a dwell time of 0.1 s were used. A standard solution of 3-methyl butanal (50 mg/L) was measured at

the beginning and at the end of the measurement of each set of samples. This procedure was used to check the reproducibility of the results. When the volatile intensity of the standard solution varied, the volatile intensity of the samples was corrected according to this variation. This external standard allowed the comparison of different sets of samples run throughout this study. Volatile intensities were expressed in arbitrary intensity units, as only relative comparisons were performed.

3.2.2.4. Differential Scanning Calorimetry (DSC)

This method was used to measure the gelatinisation properties of starches and the glass transition temperature of starchy samples.

- Principle

DSC is a dynamic thermo-analytical technique in which a sample is heated at a fixed rate, and the energy needed to heat it is recorded. In a power compensated DSC, the sample is placed in a pan; an empty pan of the same type is also placed in the machine, as represented in Figure 3.8.

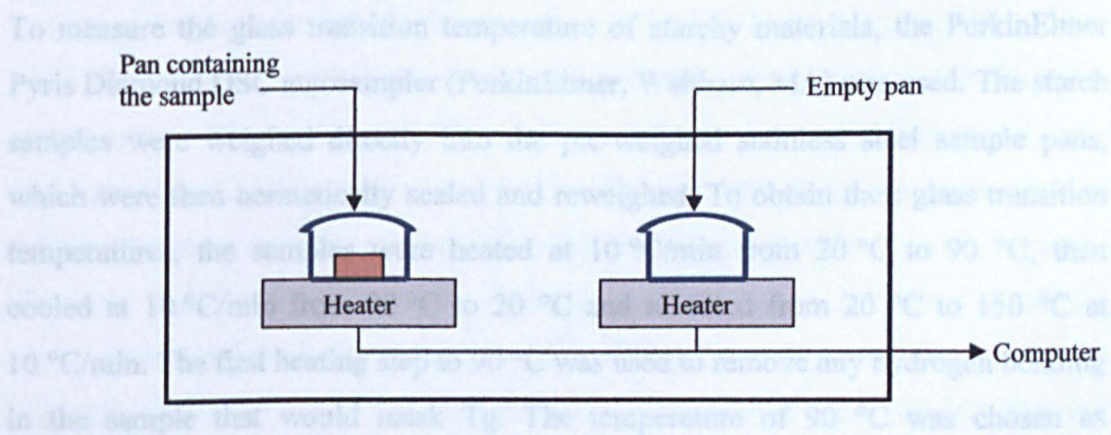


Figure 3.8: Representation of a power-compensated DSC.

Both pans are heated at the same time in the machine. The energy necessary to heat them at the same rate and maintain them at the same temperature is monitored. The energy needed might differ as the sample goes through a transition. Indeed, some transitions can be exothermic (heat is released, hence less energy is needed to keep a constant heating rate), other endothermic (heat is necessary for the transition, hence

more energy is needed to keep the heating rate constant). By comparing the energy necessary to heat an empty pan and a pan containing a sample, it is possible to visualise these transitions, such as starch gelatinisation or a glass to rubber phase transition.

- Experimental procedure

To measure the gelatinisation properties of starches, the Mettler Toledo 823e DSC coupled with the TS0801RO Mettler Toledo sample robot (Mettler Toledo, Columbus, OH) was used. The starch samples were mixed with water (starch/water ratio = approximately 1/3) directly into the pre-weighed stainless steel sample pans, which were then hermetically sealed and reweighed. All sealed samples were equilibrated at room temperature for at least 12 h before being heated at 10 °C/min from 25 °C to 110 °C in order to obtain their gelatinisation thermal profiles. Heat flow and temperature were calibrated using pure indium. Onset, peak and conclusion temperatures, as well as overall enthalpy (expressed as J/g of dry sample) associated with starch gelatinisation were determined using the Mettler Toledo STARE default DB analysis programme software (version 9.0).

To measure the glass transition temperature of starchy materials, the PerkinElmer Pyris Diamond DSC autosampler (PerkinElmer, Waltham, MA) was used. The starch samples were weighed directly into the pre-weighed stainless steel sample pans, which were then hermetically sealed and reweighed. To obtain their glass transition temperatures, the samples were heated at 10 °C/min from 20 °C to 90 °C, then cooled at 10 °C/min from 90 °C to 20 °C and reheated from 20 °C to 150 °C at 10 °C/min. The first heating step to 90 °C was used to remove any hydrogen bonding in the sample that would mask T_g. The temperature of 90 °C was chosen as temperatures above this could generate some chemical reactions (such as caramelisation) in the samples. The second heat treatment up to 150 °C was the one used to evaluate T_g. An empty pan was used as a reference to balance the heat capacity of the sample pan. Heat flow and temperature were calibrated using pure indium. Onset and end temperatures, as well as overall enthalpy (expressed as J/g of dry sample) associated with the glass transition temperature, were determined using the PerkinElmer Pyris analysis programme software (version 7.0). T_g was considered

to be the temperature corresponding to half the enthalpy of the transition (Figure 3.9).

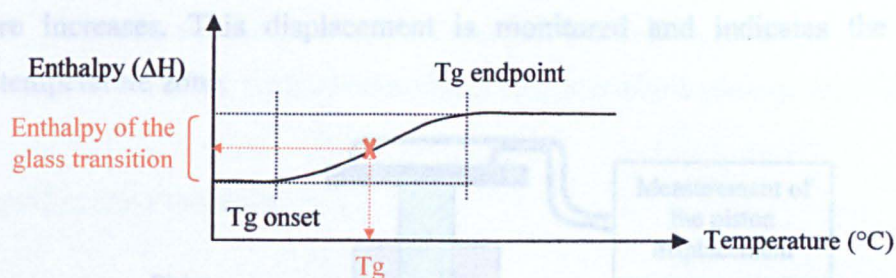


Figure 3.9: Tg determination by DSC.

3.2.2.5. Phase Transition Analyser (PTA)

The PTA was used to measure the glass transition temperature of starchy powders (model systems and breakfast cereals).

- Principle

The principle of the PTA is based on the measurement of a volume change when a system undergoes a constant pressure whilst heated. When a sample is in a glassy state, the molecules within the sample are not well ordered, hence leaving some empty spaces. When the temperature is increased, the compressibility of polymers increases (Ferry, 1970). Moreover, the sample viscosity drops when the glass to rubber transition zone is reached. This increased compressibility and decreased viscosity both lead to a system volume decrease when the glass transition temperature is reached: the molecules move, filling the empty spaces in the sample originally due to the glassy state and the particulates become more flexible allowing better packing. The PTA can determine the glass transition temperature zone by monitoring these volume changes.

The PTA consists of two sealed chambers, top and bottom, separated by an interchangeable capillary die. Before the measurement, the sample is loaded and the piston is then screwed back in place. When the measurement starts, pressure is applied: it lifts up both the bottom chamber and the slide. The sample becomes in

contact with the piston (Figure 3.10). During the measurement, the piston is displaced to maintain the pressure constant as the sample volume decreases when the temperature increases. This displacement is monitored and indicates the glass transition temperature zone.

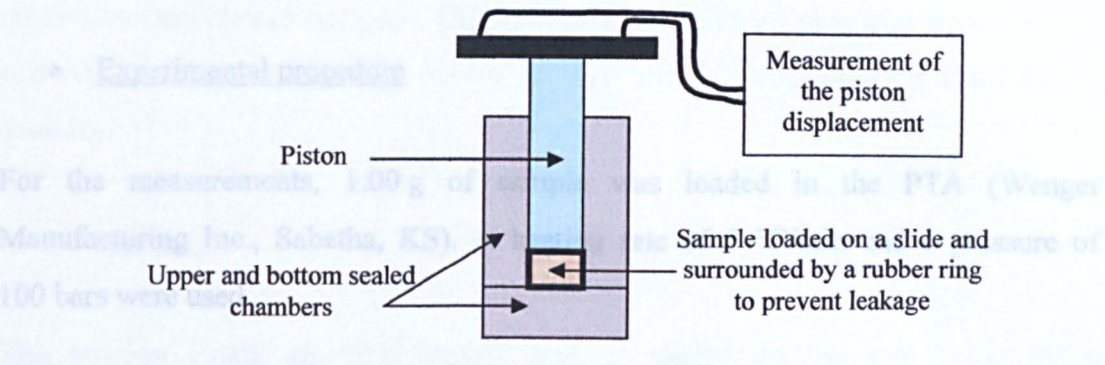


Figure 3.10: Schematic representation of a phase transition analyser.

- Glass transition temperature determination

The linear displacement of the piston, measuring the sample deformation, compaction, and flow relative to the initial sample height, is used to determine the glass transition temperature range (Plattner et al., 2008). A typical sample deformation curve and the corresponding derivative values are presented in Figure 3.11.

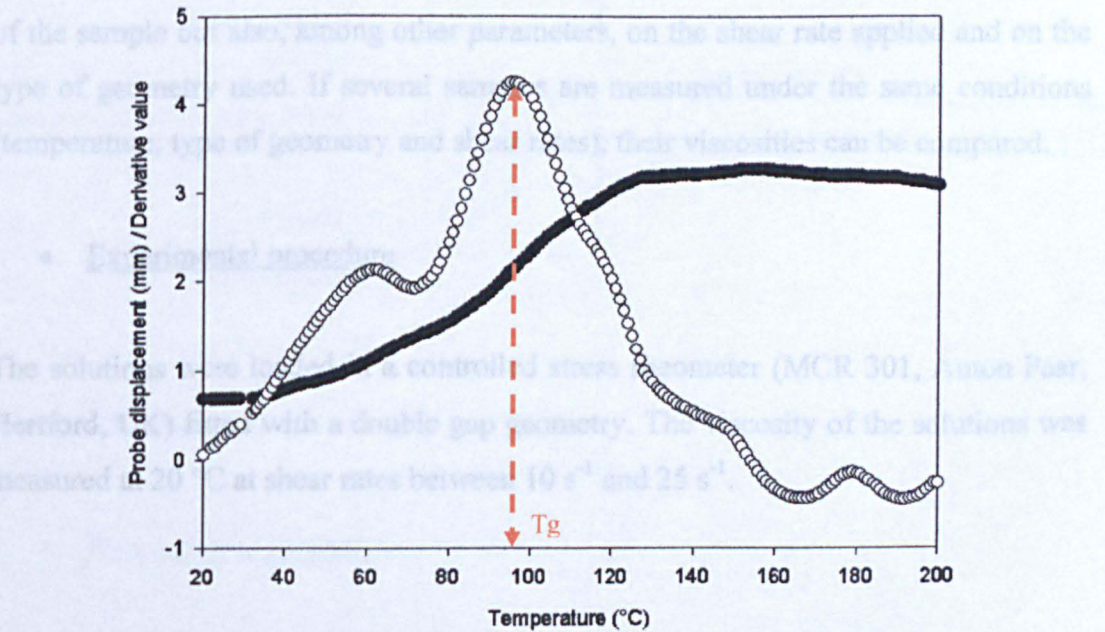


Figure 3.11: Probe displacement curve (●) and corresponding derivative values (○) of a pregelatinised starch sample with a moisture content of 8.1 % (wwb) as measured by PTA.

The glass transition temperature of a sample was considered to be the maximum data point of the derivative deformation curve, being 95 °C for the sample presented in Figure 3.11. The main advantage of a PTA compared to a DSC is the possibility to measure higher values of Tg with a better sensitivity. It is also a cheaper technique.

- Experimental procedure

For the measurements, 1.00 g of sample was loaded in the PTA (Wenger Manufacturing Inc., Sabetha, KS). A heating rate of 5 °C/min and a pressure of 100 bars were used.

3.2.2.6. *Controlled stress rheometer*

A controlled stress rheometer was used to measure the viscosity of several starch solutions.

- Principle

A rheometer measures the forces applied to a sample when a specific geometry moves while in contact with this sample. These forces are dependent on the viscosity of the sample but also, among other parameters, on the shear rate applied and on the type of geometry used. If several samples are measured under the same conditions (temperature, type of geometry and shear rates), their viscosities can be compared.

- Experimental procedure

The solutions were loaded in a controlled stress rheometer (MCR 301, Anton Paar, Hertford, UK) fitted with a double gap geometry. The viscosity of the solutions was measured at 20 °C at shear rates between 10 s⁻¹ and 25 s⁻¹.

3.2.2.7. Thermo-Gravimetric Analysis (TGA)

The TGA was used to observe the mass change of model systems or breakfast cereal products as a function of temperature, whilst the sample was subjected to a controlled temperature program. This measurement gave insights into the water loss and consequently the binding ability as well as the decomposition rates of the samples.

- Principle

The sample, inside an inert sample pan, is placed on the pan holder of a microbalance. A furnace surrounds the panned sample and microbalance pan holder and arm, as illustrated in Figure 3.12. A thermocouple is located inside the furnace, immediately underneath the holder, to monitor the change in temperature of the sample, taking into account the known lag between the measured furnace temperature and the centre of the sample support temperature. The chamber, as well as the furnace where the sample is located, can be purged with a thermally inert gas, such as nitrogen, if for example oxidation reactions must be avoided.

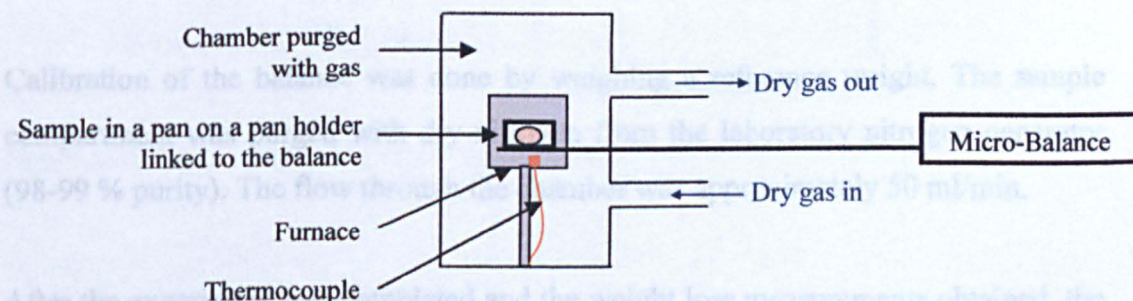


Figure 3.12: Schematic representation of the TGA.

- Experimental procedure

The samples were analysed using a TGA fitted with an autosampler (Mettler Toledo TGA/SDTA851e, Columbus, OH) operating with Mettler STARe software (version

9.0, Mettler Toledo). Wet breakfast cereal flakes ($\approx 20\%$ moisture wwb) were frozen, milled and sieved (particle size between 212 and 250 μm). The powders were then stored in hermetic glass jars and left on roller beds for several hours to ensure moisture homogeneity within the sample. The exact sample moisture content was measured using the method presented in section 3.2.2.1. Around 20 mg of sample were weighed into 100 μl aluminium pans. These pans were then hermetically sealed with lids of thin aluminium which could be pierced easily. This ensured that there would be no water loss from the sample whilst waiting on the carousel. Prior to the measurement, the lid was automatically pierced. The samples were then heated. Three heating programmes were used in this study:

- First programme: the temperature is raised at 10 $^{\circ}\text{C}/\text{min}$ from 25 $^{\circ}\text{C}$ to 190 $^{\circ}\text{C}$.
- Second programme: the temperature is raised at 10 $^{\circ}\text{C}/\text{min}$ from 25 $^{\circ}\text{C}$ to 180 $^{\circ}\text{C}$ and held at 180 $^{\circ}\text{C}$ for 10 min.
- Third programme: the temperature is raised at 10 $^{\circ}\text{C}/\text{min}$ from 25 $^{\circ}\text{C}$ to 250 $^{\circ}\text{C}$ and held at 250 $^{\circ}\text{C}$ for 10 min.

Two repetitions per sample were performed. The sample weight was automatically recorded every 20 s.

Calibration of the balance was done by weighing a reference weight. The sample compartment was purged with dry nitrogen from the laboratory nitrogen generator (98-99 % purity). The flow through the chamber was approximately 50 ml/min.

After the experiment was completed and the weight loss measurements obtained, the data were normalised to remove the influence of the initial sample weight. The following calculation was made (Equation 3.2):

$$\text{Mass ratio} = \frac{M_t}{M_{t0}}$$

Equation 3.2

With:

M_{t0} = Initial sample mass (g)

M_t = Sample mass at time t (g)

3.2.2.8. *Rapid Visco Analyser (RVA)*

The RVA was used in this study to observe the pasting properties of aqueous starch suspensions due to the gelatinisation of the starch granules during a heat treatment.

- Principle

The RVA is required to stir a sample at a controlled speed and measures the torque (force, shear stress) (Goode et al., 2005). The instrument consists of a stationary thermally controlled enclosure into which a canister containing the sample is placed (Figure 3.13). A plastic paddle is inserted into the canister and is used to stir the sample. The torque on the stirrer is measured. As the instrument can heat up the sample during the measurement, it can be used to observe the sample viscosity changes due to this heat treatment. Hence, when native starches are mixed with water, the pasting properties of the starches can be observed.

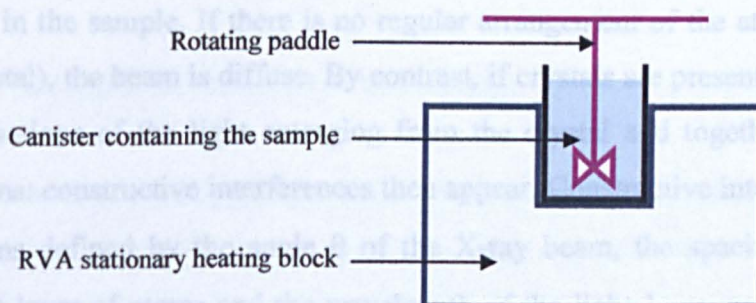


Figure 3.13: Representation of a rapid visco-analyser.

- Experimental procedure

Before initiating a sample measurement, the paddle was placed in the torque measuring arm of the RVA (Newport Scientific, Warriewood, Australia) and zeroed at 160 rpm against air. After pre-weighing 4.00 g of sample (dry-matter) and adjust the weight to a total of 28.00 g with distilled water into the aluminium canisters, the sample was immediately placed into the RVA block. For all samples, an initial 10 s mixing period at a constant rate (960 rpm) was carried out in order to ensure the homogeneity of the suspension. For the remainder of the rheological measurement

duration, a constant stirring speed of 160 rpm was applied and the apparent viscosity was measured. A 25 °C plateau was applied at first for 2 min. The temperature was then increased from 25 °C to 95 °C at a constant rate of 14 °C/min. The temperature of 95 °C was held for 180 s and was then decreased from 95 °C to 25 °C at a constant rate of 14 °C/min. In all investigations, measurement points were taken every 4 s.

3.2.2.9. *Wide Angle X-ray Scattering (WAXS)*

X-ray scattering is a diffraction method that was used to study the crystallinity of polymers, i.e. starch, but also to detect NaCl crystals.

- Principle

The general principle of diffraction methods is based on the phenomenon of interference. When a sample is exposed to X-rays, the beams of the X-rays penetrate it. The X-rays are then scattered by interaction with the electrons of the atoms present in the sample. If there is no regular arrangement of the atoms in the sample (no crystal), the beam is diffuse. By contrast, if crystals are present in the sample, the wave motions of the light emerging from the crystal add together in only certain directions: constructive interferences then appear. Constructive interferences occur in directions defined by the angle θ of the X-ray beam, the spacing d between two adjacent layer of atoms and the wavelength of the light λ , as represented in Figure 3.14.

If a crystal particle was present in the sample, but did not have a spacing (d) which was in the same order of magnitude as the wavelength of the X-ray, simple reflections and scattering of the X-rays would occur (Barrow, 1961). When all the conditions are present to generate constructive interferences, a sharp peak is obtained on the X-ray pattern measured by the equipment. These sharp peaks indicate the presence of specific crystals in the sample. When no crystals are present in the sample, a diffuse X-ray pattern is nevertheless obtained.

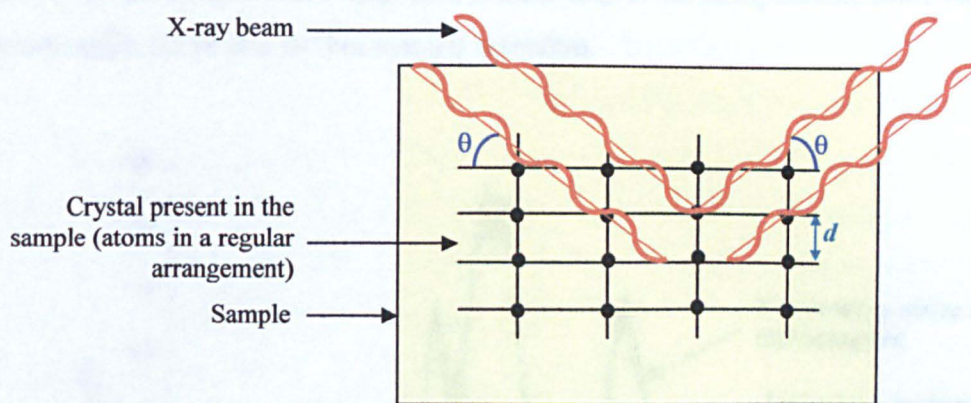


Figure 3.14: Representation of constructive interferences occurring when X-ray beams are striking two regularly arranged layers of atoms (●) (Barrow, 1961).

In the case of starch, both crystalline and amorphous materials are present. The X-ray pattern obtained is then the superposition of a diffuse pattern (amorphous material) and sharp peaks (due to the presence of crystals), as presented in Figure 3.15.

- Calculation of the degree of crystallinity

The method described by Hermans and Weidinger (1948) is a technique widely used to measure the crystallinity of a sample. This crystallinity measurement method involves calculating the area under the entire X-ray pattern (crystalline + amorphous regions) and the area under the peaks only (crystalline regions), as presented in Figure 3.15.

The relative crystallinity of the sample is then given by the Equation 3.3:

$$\text{Crystallinity} = \left(\frac{\text{Crystalline scattering area}}{\text{Total scattering area}} \right)$$

Equation 3.3

In this study, the calculation of the area under the curve was done using the software Eva (version 5, Diffrac plus, Bruker AXS, Karlsruhe, Germany). The area of the amorphous background was manually selected based on the X-ray pattern of fully amorphous starches from the same botanical origins. However, even though all the

sample's calculations were done in a similar way to be comparable, some calculation errors might occur due to this manual selection.

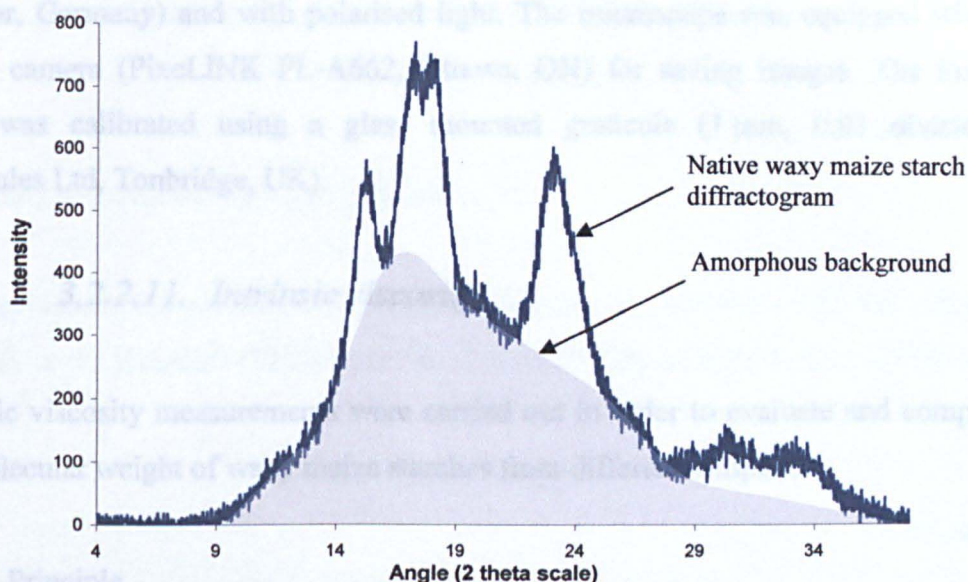


Figure 3.15: X-ray diffractogram of native waxy maize starch showing the sample's crystalline scattering region (in white) and the amorphous scattering region (in grey).

- Experimental procedure

The X-ray patterns of the starches were obtained using a Bruker-AXS D5005 diffractometer (Siemens, Erlanger, Germany) with 2.2 kW sealed copper source operating at 40 mA and 40 kV. The scanning region of the diffraction angle (2θ) ranged from 4 to 38°. The step size and the time per step varied depending on the study. Values that were used for a specific study are mentioned in the corresponding chapter. The samples were 1 mm thick with a smooth surface and the sample rotation speed was 60 rpm.

3.2.2.10. Optical microscopy

Optical microscopy was used to visualize starch materials. When viewed under polarised light, all native starch granules appear to shine while exhibiting a dark “Maltese cross”. This phenomenon is known as birefringence.

A few milligrams of native starch samples were mixed into one or two drops of water deposited on a standard glass microscope slide. The mixture was then covered with a glass coverslip. The samples were visualised with a microscope (Leitz Diaplan, Wetzlar, Germany) and with polarised light. The microscope was equipped with a digital camera (PixeLINK PL-A662, Ottawa, ON) for saving images. The image scale was calibrated using a glass mounted graticule (1 mm, 0.01 divisions; Graticules Ltd, Tonbridge, UK).

3.2.2.11. *Intrinsic viscosity*

Intrinsic viscosity measurements were carried out in order to evaluate and compare the molecular weight of waxy maize starches from different samples.

- Principle

The intrinsic viscosity of a polymer solution is the extrapolation of the solution viscosity to a polymer concentration $c = 0$. The extrapolation is done by measuring the viscosity of several solutions with different polymer concentrations and performing the following calculations for each sample:

- The relative viscosity η_{rel} of a sample is first calculated. This is the ratio of the solution viscosity η , to that of the solvent, η_s :

$$\eta_{rel} = \frac{\eta}{\eta_s}$$

Equation 3.4

- From the relative viscosity, the specific viscosity η_{sp} is calculated:

$$\eta_{sp} = \eta_{rel} - 1$$

Equation 3.5

- The reduced viscosity η_{red} and the inherent viscosity η_{inh} can then be calculated as follows, with c the polymer concentration (g/ml):

$$\eta_{red} = \frac{\eta_{sp}}{c} \quad \eta_{inh} = \frac{(\ln \eta_{rel})}{c}$$

Equation 3.6 and 3.7

The limit at $c \rightarrow 0$ of both η_{red} and η_{inh} is defined as the intrinsic viscosity $[\eta]$, presumably so named because it is an intrinsic function of the dissolved / dispersed macromolecule (Harding, 1997). The dependence of η_{red} and $[\eta]$ is described by the Huggins (1942) equation (Equation 3.8), with K_H being the Huggins constant:

$$\eta_{\text{red}} = [\eta](1 + K_H[\eta].c)$$

Equation 3.8

According to Equation 3.8, $\eta_{\text{red}} = [\eta]$ when $c = 0$.

The equivalent concentration dependence relation of Equation 3.8 for inherent viscosity is the Kraemer (1938) equation (Equation 3.9), with K_K being the Kraemer constant:

$$\eta_{\text{inh}} = [\eta](1 - K_K[\eta].c)$$

Equation 3.9

As previously, $\eta_{\text{inh}} = [\eta]$ when $c = 0$.

From the viscosity measurement of several solutions with different polymer concentrations, the inherent and reduced viscosity of each solution can be calculated. A graph, such as the one presented in Figure 3.16, can then be obtained and the polymer intrinsic viscosity evaluated:

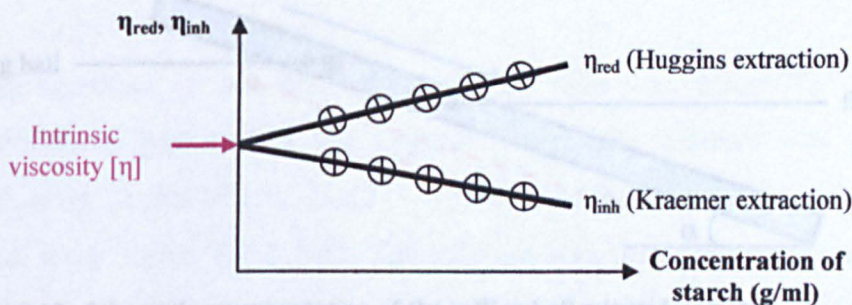


Figure 3.16: Huggins and Kraemer extraction methods for intrinsic viscosity (Harding, 1997).

From the intrinsic viscosity results, the molecular weight average of a polymer (M) can be estimated using the Mark Houwink equation (Equation 3.10):

$$[\eta] = K'.M^a$$

Equation 3.10

K' and a are constants depending on the molecular conformation of the polymer. According to Harding (1997), there are 2 molecular contributions to the intrinsic viscosity, one from the shape and the other from the size of the polymer. Hence, for a specific type of polymer, the intrinsic viscosity is influenced only by its molecular weight.

- Experimental procedure

The starch samples were mixed with aqueous solutions of KOH (5 M) at concentrations of 200 or 400 mg of starch/ml of mixture and stirred until complete dissolution of the starchy sample. The resulting solutions were then serially diluted in distilled water. The dilutions used will be mentioned in the corresponding chapter.

The viscosity of the diluted solutions of starch was then determined using an automated rolling ball microviscometer (AMVn, Anton Paar, Graz, Austria). This equipment estimates the viscosity of a solution by measuring the time it takes for a ball to roll between a fixed distance (d) in a glass tube filled with the sample as shown in Figure 3.17. This time varies depending on the solution viscosity and on the inclination angle (α).

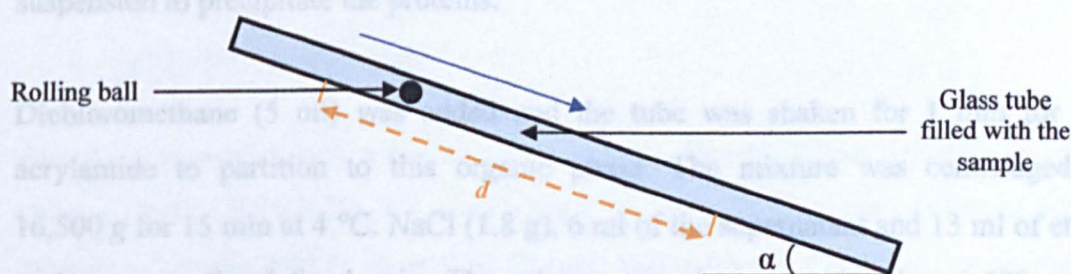


Figure 3.17: Schematic representation of the rolling ball microviscometer.

The glass tube used had a capillary diameter of 1.6 mm. The instrument temperature was controlled at 20.0 °C with a Peltier thermostat. Viscosity values were calculated using the viscometer software (Anton Paar VisioLab for AMVn, version 1.63) which utilized the rolling time of the ball between two reference positions along the tube, the tube angle, and the density of the ball and of the sample solution as input data.

Calibration of the machine was made using this glass tube filled with distilled water and for the angles used in this study.

3.2.3. Acrylamide content determination

Acrylamide content was measured in both model systems and breakfast cereals. The procedure used was the same regardless of the system and consists in two main steps. At first, the acrylamide is extracted from the system, followed by the quantification of acrylamide in the extract. The measurements were all performed by Nestle QA Centre in Germany.

- Acrylamide extraction

A powdered sample (2.0 g of model or breakfast cereal) was weighed into a 50 ml centrifuge tube and mixed with 10 ml of water at 60 °C and 100 µl of 5 µg/ml [$^{13}\text{C}_3$]-acrylamide solution (internal standard). The suspension was homogenized (Ultra-Turrax) for 1 min. Carrez I (potassium hexacyanoferrate(II)-3-hydrate, 150 g/l) and II (zinc sulfate-7-hydrate, 300 g/l) solutions (1 ml of each) were then added to the suspension to precipitate the proteins.

Dichloromethane (5 ml) was added and the tube was shaken for 1 min for the acrylamide to partition to this organic phase. The mixture was centrifuged at 16,500 g for 15 min at 4 °C. NaCl (1.8 g), 6 ml of the supernatant and 13 ml of ethyl acetate were stirred for 1 min. The mixture was then centrifuged at 1,500 g for 15 min at 4 °C. The organic phase was transferred in a vial with 2 ml of water and shaken before evaporating the ethyl acetate under a nitrogen stream. Two further extractions of the 6 ml aqueous solution with ethyl acetate were conducted by pooling successively the ethyl acetate phases together in the vial. In total, three extracts of 2 ml were obtained.

In the above sequence, the ethyl acetate acts as a second organic compound used to extract the acrylamide. The NaCl was added to help the migration of the acrylamide

to the organic phase (salting-out effect). These successive steps were used to maximise the extraction of this compound.

Isolute multimode cartridges (International Sorbent Technology, Tucson, AZ) were preconditioned with 3 ml of methanol and 6 ml of water. The acrylamide extract (2 ml) was then loaded onto the cartridge and eluted with 1 ml of water. This procedure was repeated for each extracts of 2 ml. The resulting 3 ml (3 x 1 ml of water used for acrylamide elution) were concentrated to 500 µl under a nitrogen stream. The extract was filtered through a 0.2 µm pore size filter to remove any solid impurities. An aliquot (180 µl) was mixed with 90 µl of methanol and the mixture was analyzed by liquid chromatography coupled with electrospray ionization tandem mass spectrometry (LC-ESIMS/MS) (Quattro, Waters, Saint-Quentin-En-Yvelines, France).

In the above section, the isolute multimode cartridges were used to perform a solid / liquid extraction. Indeed, acrylamide has a good affinity for this type of cartridge and hence, was retained. This extraction was used to separate acrylamide from any other compounds which could have been extracted by the organic phases. As acrylamide is also water soluble, water allowed the elution of the compound from the cartridge.

- LC-ESIMS/MS analysis

Analytical separation was achieved with a column (Shodex RSpak DE-413L + guard column Shodex RSpak DE-SG, New York City, NY). Elution was performed with a solution of formic acid in water 0.01 % (v/v) and methanol (90/10 v/v respectively) with a flow rate of 0.3 ml/min. Injection volume was 60 µl. The ionization mode used was positive. The MS detector was set in the selected reaction monitoring mode to produce the following daughterions: 72→55, 72→54, 72→27 for acrylamide and 75→58, 75→29 for [¹³C₃]-acrylamide (collision energy: 11 to 18 eV, inter channel delay 0.02 s, inter scan delay 0.1 s). The needle voltage was set to 3.2 kV and the cone voltage to 22 V. The dwell time was 0.2 s. The source temperature was 100 °C and the volatilisation temperature 350 °C. The nebuliser gas flow rate was 100 l/h. Calculation of acrylamide content was done by measuring the ratio between peak areas of acrylamide and the internal standard.

3.2.4. Statistical analyses

All the experimental designs in this study were created using an experimental design software (Design Expert, version 6.0.6, Stat-Ease Inc., Minneapolis, MN) utilising the D-optimal response surface option. When a set of samples followed an experimental design, the resultant data were analysed using this same software to determine the significance of the results (analysis of variance ANOVA, statistical significance established at $p < 0.05$).

However, when a specific study did not follow a Design Expert experimental design, analysis of variance was performed using SPSS software (version 15.0, SPSS Inc., Chicago, IL). Significant differences among means of treatments were evaluated by the post hoc multiple comparisons Duncan test with statistical significance established at $p < 0.05$.

4. Model system development

4.1. Introduction

From the literature available on the influence of NaCl on starch, it seemed that this salt may interact with the native starch granules during the mixing step (see section 2.3.2.2). It may also influence starch gelatinisation (section 2.3.3.2). Literature on Maillard and caramelisation reactions seemed to indicate that NaCl could possibly lead to differences in product colour, aroma and flavour by influencing either directly the chemistry of the reactions or indirectly by modifying the environment of the reaction during the toasting step (section 2.4.2). It might also enhance a possible alteration of the starch molecules during this severe heat treatment (section 2.4.3).

It was observed during preliminary trials done before the beginning of this study that removing NaCl from breakfast cereal products was mostly noticeable after the toasting stage. Indeed, no differences were observed during ingredient mixing regardless of the NaCl level used. After the cooking step, the stickiness of the breakfast cereal dough was slightly affected by the level of NaCl used in the product, but the stickiness could be corrected by adjusting the level of water. However, during the flake toasting stage, the absence of NaCl had several repercussions on the products which could not be corrected. It gave products with a less salty taste, and the overall flavour of the product was also affected (bland taste). Lowering the NaCl affected the colour of breakfast cereals. Changing the toasting times and/or temperatures were tried to adjust colour and flavour formation in the product. However, even though the colour could be adjusted by such modifications, the toasted flavour of the product was altered and it did not compensate for the bland taste. Therefore, it seemed that the main negative impacts due to lowering NaCl in breakfast cereals occurred during the toasting step. Hence, observing the influence of NaCl on breakfast cereal products during such a heat treatment was the first priority of this study.

These preliminary observations on the influence of NaCl on product overall flavour and colour suggest that this ingredient might have an influence on Maillard and/or caramelisation reactions during the toasting step of the products. To be able to observe the impact of NaCl on these reactions, some reaction markers had first to be selected. Indeed, Maillard and caramelisation reactions generate hundreds of components, which make these reactions difficult to follow as a whole. The reaction markers chosen were colour and several residual volatile molecules formed in breakfast cereal products, called from now on the “volatiles of interest”. Once the markers were selected, a model system mimicking breakfast cereals for these specific markers was developed.

Developing a model system for breakfast cereal products was needed as it takes one day to produce only 2 batches of product. Moreover, the complexity of the food system would make the determination of the exact impact of NaCl on the variation of a specific attribute difficult. Hence, a model system would give some flexibility towards the number of samples made and the number of variables studied. Moreover, the influence of NaCl would be easier to pinpoint in a model system containing less components.

4.2. Aims

To observe the impact of NaCl on Maillard and/or caramelisation reactions, the reaction markers chosen were colour and the residual “volatiles of interest” formed (molecules still present in the system, but which can be released into the headspace when the product is hydrated). Hence, the aim of the model system development was to obtain a system mimicking breakfast cereal products regarding these specific markers. At first, the markers chosen were measured in breakfast cereal products. Several model systems using the primary components of breakfast cereals were then made. Colour and residual volatile molecules of the models were analysed and compared to breakfast cereals. The model system composition was then adjusted in order to match the breakfast cereals’ markers.

4.3. System preparation

Some model systems were prepared by mixing the components presented in Table 4.1 and Table 4.2 using the procedure explained in section 3.2.1.1. After drying, the powder obtained was a homogeneous blend of native starch, amino-acids and glucose, which moisture content was adjusted to 20 % \pm 1 % (wwb). For both types of model systems, aliquots of 15 g were heated at 230 °C for 0, 5, 10, 15 and 25 min.

- Model system containing native starch, glucose and glycine

Table 4.1: Model system composition before drying (glucose/glycine molar ratio of 1/1).

Model components	Weight (g per 100g)
Native waxy maize starch	49.300
Glycine	0.990
D-Glucose	0.410
Distilled water	49.300
Total	100.000

- Model system containing native starch, glucose and a cocktail of amino-acids

Table 4.2: Model system compositions before drying (models containing various molar ratios of amino-acids, see section 4.4.3, with a constant amino-acid cocktail / glucose molar ratio = 1/1).

	Model system #1	Model system #2	Model system #3
Native waxy maize starch (g)	49.175	49.134	49.178
Leucine (g)	0.381	0.147	0.178
Valine (g)	0.188	0.054	0.160
Alanine (g)	0.061	0.032	0.122
Glutamic acid (g)	0.037	0.516	0.201
D-Glucose (g)	0.983	0.983	0.983
Distilled water (g)	49.175	49.134	49.178
Total (g)	100.000	100.000	100.000

Wheat, wheat and rice mixture, corn and rice final breakfast cereal products were also used in this part of the study (see section 3.2.1.5).

4.4. Results and discussion

4.4.1. Colour and residual volatile molecules for breakfast cereals

Compounds from breakfast cereals were released into the headspace by the addition of water and measured according to the method given in section 3.2.2.3. The volatile molecules released from final mixture of wheat and rice breakfast cereal products are presented in Figure 4.1.

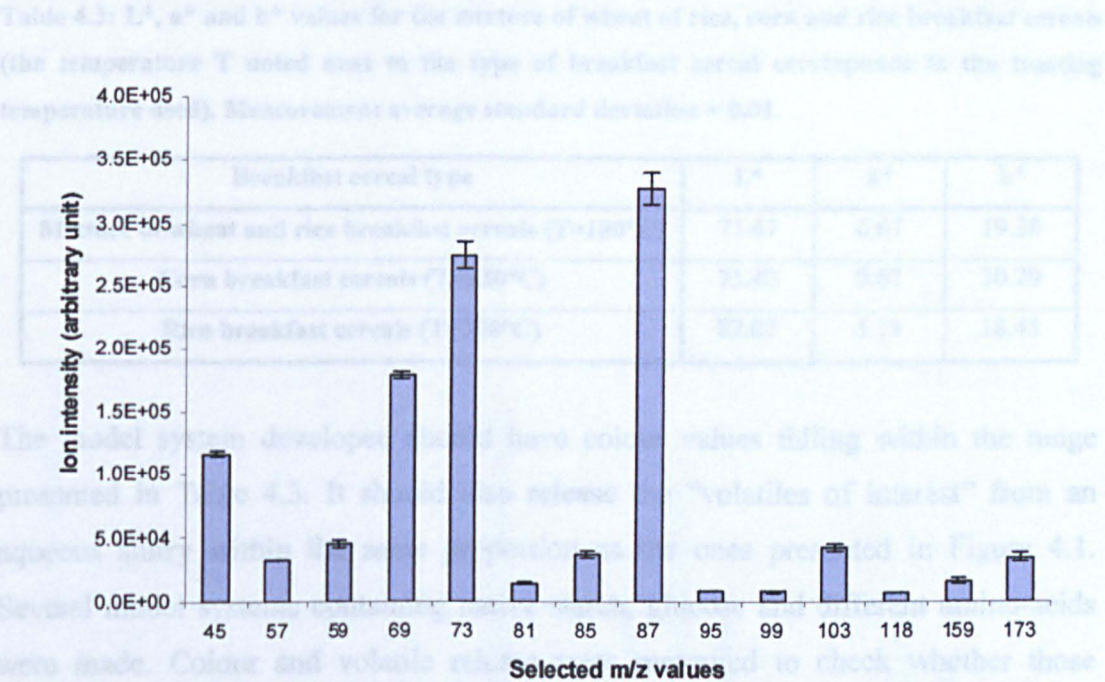


Figure 4.1: Headspace analysis of the most intense residual volatile compounds released from mixture of wheat and rice breakfast cereal products versus the mass / charge ratio as measured by APCI-MS (after full scan) (n=3 measurements).

4.4.2. Model system containing glycine

Only the volatile residual molecule pattern for the mixture of wheat and rice flakes is presented as similar patterns were obtained for puffed rice and corn flakes (data not shown). Hence, one may assume that these volatiles were not specific to a breakfast cereal recipe but were common to all breakfast cereal products. The 6 most intense volatiles released had molecular weights of 44, 58, 68, 72, 86 and 102 g/mol (data derived from the protonated molecular ion data from APCI-MS analysis) and correspond to what was called the “volatiles of interest”. Therefore, in order to match breakfast cereal products regarding volatile release, it was decided that the model

system being developed should contain these 6 types of volatiles in a ratio similar to those found for breakfast cereal products. It is recognised that these may not be the key compounds for flavour which contribute to quality, but they will act as general markers for the reactions.

The colour of the mixture of wheat and rice, corn and rice breakfast cereal products, as measured according to section 3.2.2.2, is presented in Table 4.3.

Table 4.3: L*, a* and b* values for the mixture of wheat of rice, corn and rice breakfast cereals (the temperature T noted next to the type of breakfast cereal corresponds to the toasting temperature used). Measurement average standard deviation = 0.01.

Breakfast cereal type	L*	a*	b*
Mixture of wheat and rice breakfast cereals (T=180°C)	71.47	6.67	19.38
Corn breakfast cereals (T=230°C)	75.42	9.67	30.20
Rice breakfast cereals (T=200°C)	82.07	5.73	18.43

The model system developed should have colour values falling within the range presented in Table 4.3. It should also release the “volatiles of interest” from an aqueous slurry within the same proportion as the ones presented in Figure 4.1. Several model systems containing native starch, glucose and different amino-acids were made. Colour and volatile release were measured to check whether those models fitted the above requirements.

4.4.2. Model system containing glycine

A model system containing native starch, glucose and glycine was made as an initial attempt. The amino acid glycine was chosen for its ability to develop colour via Maillard reactions. The composition of the model was presented in Table 4.1. The glycine /glucose molar ratio (1/1) was chosen based on the publication by Tehrani et al. (2002).

The preparation of the model system was established in order to mimic some of the steps used during breakfast cereal preparation. Indeed, every component of the model

system was mixed to obtain a visually homogeneous suspension. At that stage, the interactions between the model system components could mimic those occurring during the ingredient mixing stage of breakfast cereals. Moreover, it insured an effective distribution of the powders within the mix. The model system suspension was then dried and re-hydrated to a moisture content of 20 % (wwb). This moisture content was that of wet flakes, before the toasting stage. The model was then heated in an oven to mimic the toasting stage of breakfast cereals.

After the heat treatment of the model system at 230 °C for times varying between 0 and 25 min, the residual molecules analysis was performed. The results are presented in Figure 4.2.

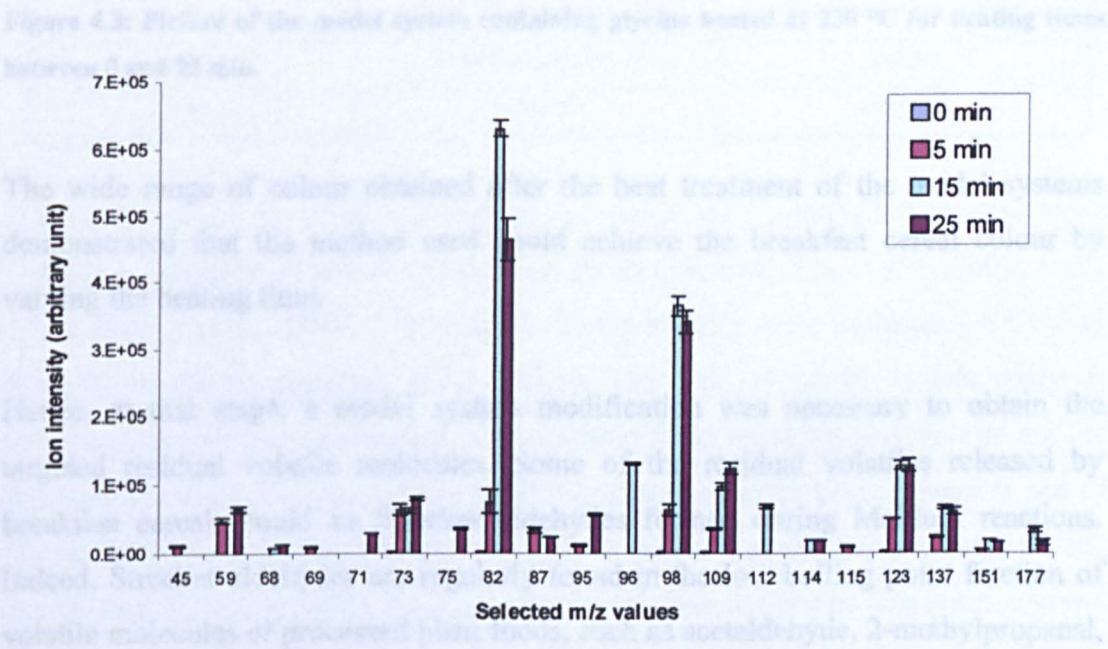


Figure 4.2: Headspace analysis of the most intense residual volatile compounds released from the model system containing native starch, glucose and glycine versus their mass / charge ratio and their heating time at 230 °C as measured by APCI-MS (after full scan). The error bars correspond to \pm one standard deviation (n=3 samples with 3 measurements per sample).

The most intense residual volatiles released by the model system containing native starch, glucose and glycine had molecular weights of 72, 81, 97, 108 and 122 g/mol according to the APCI-MS analysis. It can be considered that the main volatiles released were the same regardless of the heating time (except for a heating time of 0 min where no volatiles were released as Maillard reactions did not occur in the absence of heat). The residual volatiles released by this model system after heating

were different from those released from breakfast cereal products (Figure 4.1 and Figure 4.2). Therefore, this type of model system did not match breakfast cereals regarding this marker.

The colour of the model systems after the heat treatment can be observed in Figure 4.3.

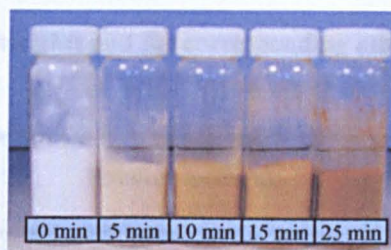


Figure 4.3: Picture of the model system containing glycine heated at 230 °C for heating times between 0 and 25 min.

The wide range of colour obtained after the heat treatment of the model systems demonstrated that the method used could achieve the breakfast cereal colour by varying the heating time.

Hence, at that stage, a model system modification was necessary to obtain the targeted residual volatile molecules. Some of the residual volatiles released by breakfast cereals could be Strecker aldehydes formed during Maillard reactions. Indeed, Strecker aldehydes are regularly found in the low boiling point fraction of volatile molecules of processed plant foods, such as acetaldehyde, 2-methylpropanal, 2-methylbutanal and 3-methylbutanal (Kolek et al., 2006; Cremer and Eichner, 2000). Being volatile, the aldehydes formed during the Strecker degradation have often been thought to be important contributors to the aroma of foodstuffs. Many patents have been granted which use the Strecker degradation to produce flavouring materials of various types, such as, maple, chocolate, coffee, tea, honey, mushroom and bread (O'Brien et al., 1998).

The molecular weight of Strecker aldehydes is associated to the molecular weight of the amino-acid it is derived from according to the reaction shown in Figure 2.15. Based on the hypothesis that the volatiles could be Strecker aldehydes, the molecular weight of the volatile can be calculated from the amino-acid (Table 4.4). Glutamic

acid, leucine (or isoleucine), valine and alanine could be the amino-acids reacting to form the volatile molecules released from breakfast cereals and having molecular weights of 102, 86, 72, and 44 g/mol respectively. Both leucine and isoleucine could lead to the formation of the volatile with a weight of 86 g/mol as both these amino-acids have the same molecular weight.

Table 4.4: Volatile molecular weights and the corresponding amino-acid it could derive from via Strecker degradation.

Volatile molecular weight (g/mol)	Possible molecule	Amino-acid from which this molecule may derive
102		Glutamic acid
86	2 or 3-methyl butanal	Leucine or Isoleucine
72	2-methyl propanal	Valine
44	Ethanal	Alanine

It was previously reported that 2-methylpropanal and 3-methylbutanal are usual volatile compounds of heated cereal based foods (Vanruth et al., 1995; Collin et al., 1995; Buttery et al., 1994). However, to clearly identify these molecules, a GC-MS analysis would be required. Such analysis was not performed in this study as knowing the exact molecules that were released from breakfast cereals was not the focus of this work. Indeed, these residual volatiles were collectively chosen as one of the markers of the Maillard reactions but their link to any sensory properties is not established. Hence, knowing the exact identity of these molecules was not necessary. However, to extend our understanding of the potential role of amino-acids, these 4 amino acids (leucine, valine, alanine and glutamic acid) were investigated as a cocktail of amino-acids in the model systems, in combination with glucose and starch.

4.4.3. Model systems containing a cocktail of amino-acids

Three different levels of the above amino-acids (different ratios) were tried in model systems. This was based on the assumption that the “volatiles of interest” could be Strecker aldehydes and the main amino-acids generating these volatiles being

leucine, valine, alanine and glutamic acid. The preparation method for these systems (section 3.2.1.1) was the same as the one used for the model containing glycine. In each model system, a glucose / amino acid molar ratio of 1/1 was used. The model systems differed from each other as the molar ratio within the amino-acid cocktail was different in each system (composition presented in Table 4.2). The selected ratios are designed to reflect the levels in systems quantified to date, namely:

- System #1: amount of amino acids proportional to the supposedly corresponding Strecker aldehyde amount measured in breakfast cereal products (Figure 4.1) (Leu 53.2 %, Val 29.5 %, Ala 12.6 %, Glu 4.7 % on a molar basis)
- System #2: amount of amino acids proportional to the amount found in wheat according to Acquistucci et al. (1995) (Leu 20.7 %, Val 8.4 %, Ala 6.6 %, Glu 64.3 % on a molar basis)
- System #3: all amino-acids in the same proportions (Leu 25 %, Val 25 %, Ala 25 %, Glu 25 % on a molar basis)

After a heat treatment of the models at 230 °C between 0 and 25 min, the residual volatile molecules released were measured by APCI-MS. The results are presented in Figure 4.4.

The model system #1 (where the quantity of amino acids was proportional to the quantity of supposedly Strecker aldehyde produced) contained a very large amount of higher molecular weight molecules compared to that measured for breakfast cereals. Moreover, the observed intensity of the molecules having a weight of 58 g/mol was much higher than those of 86, 72 and 68 g/mol, which were the main molecules measured for the cereals. Hence, the proportion of the residual volatile molecules was different from the cereals. Therefore, the model system #1 was not appropriate to mimic the food product. If the volatiles that were released were indeed Strecker aldehydes, this experiment also demonstrated that the amount of a certain amino acid type was not proportional to the amount of corresponding aldehyde generated during Strecker degradation.

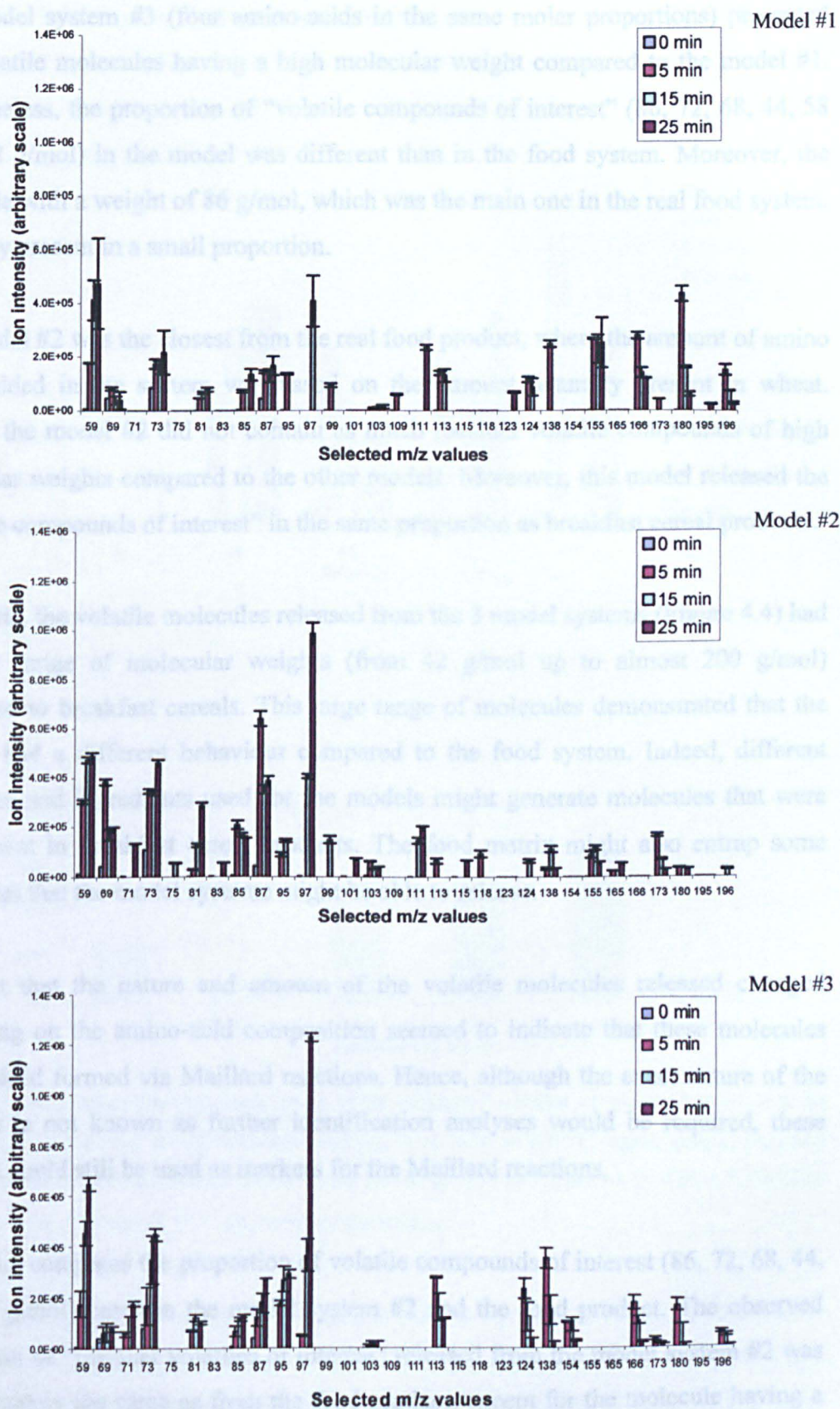


Figure 4.4: Headspace analysis of residual volatile compounds released from the model systems (1, 2 and 3) after several heating time (between 0 and 25 min) at 230 °C. The error bars correspond to ± one standard deviation (n=3 samples with 3 measurements per sample).

The model system #3 (four amino-acids in the same molar proportions) presented less volatile molecules having a high molecular weight compared to the model #1. Nevertheless, the proportion of “volatile compounds of interest” (86, 72, 68, 44, 58 and 102 g/mol) in the model was different than in the food system. Moreover, the molecule with a weight of 86 g/mol, which was the main one in the real food system, was only present in a small proportion.

The model #2 was the closest from the real food product, where the amount of amino acids added in the system was based on the amount naturally present in wheat. Indeed, the model #2 did not contain as much residual volatile compounds of high molecular weights compared to the other models. Moreover, this model released the “volatile compounds of interest” in the same proportion as breakfast cereal products.

In general, the volatile molecules released from the 3 model systems (Figure 4.4) had a wider range of molecular weights (from 42 g/mol up to almost 200 g/mol) compared to breakfast cereals. This large range of molecules demonstrated that the models had a different behaviour compared to the food system. Indeed, different processes and ingredients used for the models might generate molecules that were not present in breakfast cereal products. The food matrix might also entrap some molecules that the model systems might be able to release.

The fact that the nature and amount of the volatile molecules released changed depending on the amino-acid composition seemed to indicate that these molecules were indeed formed via Maillard reactions. Hence, although the exact nature of the volatiles is not known as further identification analyses would be required, these volatiles could still be used as markers for the Maillard reactions.

Figure 4.5 compares the proportion of volatile compounds of interest (86, 72, 68, 44, 58, 102 g/mol) between the model system #2 and the food product. The observed proportion of “residual volatiles of interest” released from the model system #2 was approximately the same as from the food product, except for the molecule having a molecular weight of 58 g/mol. This proportion may vary in the model system depending on the heating time but on average, the molecule order from the most to the least intense was $86 > \underline{58} > 72 > 68 > 102 > \underline{44}$ g/mol (Figure 4.5). This order

slightly varied from the food system (underlined molecular weights): $86 > 72 > 68 > 44 > 58 > 102$ g/mol (Figure 4.1).

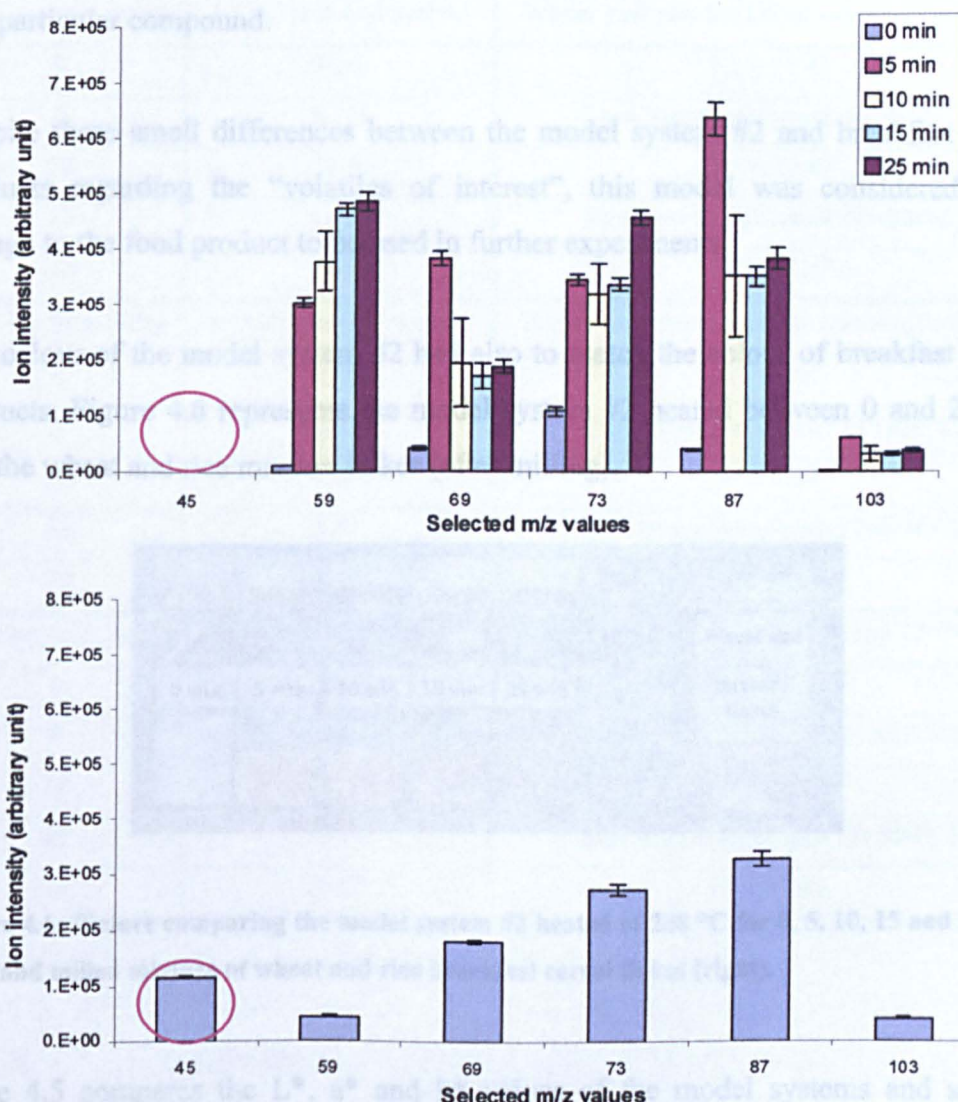


Figure 4.5: Headspace analysis comparison of the residual “volatiles of interest” released from the model system #2 for several heating times (up to 25 min) at 230 °C (upper graph, n=3) and the mixture of wheat and rice breakfast cereal (lower graph) as measured by APCI-MS (SIM). The error bars correspond to ± one standard deviation.

The molecule having a weight of 44 g/mol was not generated in any of the model systems. At first, the assumption was that this molecule was formed during Strecker degradation occurring between a reductone and the amino acid alanine. Different hypotheses can explain the absence of this molecule in the model systems. Maillard reactions might be orientated in a different way in the model systems compared to the food product. Reactions involving alanine might have a higher affinity for

another pathway, generating molecules with other molecular weights than 44 g/mol. Another explanation could be that this molecule was not formed during Strecker degradation. Therefore, adding alanine to the system does not affect the generation of this particular compound.

Despite these small differences between the model system #2 and breakfast cereal products regarding the “volatiles of interest”, this model was considered close enough to the food product to be used in further experiments.

The colour of the model system #2 had also to match the colour of breakfast cereal products. Figure 4.6 represents the model system #2 heated between 0 and 25 min and the wheat and rice mixture flakes (after milling).

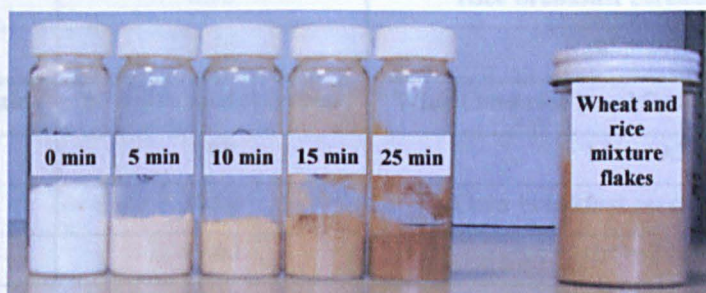


Figure 4.6: Picture comparing the model system #2 heated at 230 °C for 0, 5, 10, 15 and 25 min (left) and milled mixture of wheat and rice breakfast cereal flakes (right).

Table 4.5 compares the L^* , a^* and b^* values of the model systems and several breakfast cereal products. The L^* value of the model systems decreased with heating time at 230 °C as the system was getting darker, the a^* value increased with heating time while the b^* value increased up to a heating time of 15 min and then decreased. The L^* , a^* and b^* values of breakfast cereals depended on the type of cereal used. It was observed that the colour values of breakfast cereals were within the range obtained for the model systems heated between 5 and 15 min, except for the a^* and b^* values of corn breakfast cereals. However, the colour of the model system #2 was considered close enough to the colour of breakfast cereals to be used to study the influence of NaCl on colour development.

Table 4.5: L*, a* and b* value comparison between the model system #2 and wheat and rice mixture breakfast cereals (the temperature T noted next to the type of breakfast cereal corresponds to the toasting temperature used). Average measurement standard deviation = 0.01.

Heating time (min)	L* value model system	Wheat and rice breakfast cereals (T=180°C)
0	99.42	L* = 71.47
5	86.13	Corn breakfast cereals (T=230°C)
10	74.70	L* = 75.42
15	65.71	Rice breakfast cereals (T=200°C)
25	51.79	L* = 82.07
Heating time (min)	a* value model system	Wheat and rice breakfast cereals (T=180°C)
0	-0.47	a* = 6.67
5	2.57	Corn breakfast cereals (T=230°C)
10	5.06	a* = 9.67
15	6.35	Rice breakfast cereals (T=200°C)
25	7.48	a* = 5.73
Heating time (min)	b* value model system	Wheat and rice breakfast cereals (T=180°C)
0	3.97	b* = 19.38
5	16.02	Corn breakfast cereals (T=230°C)
10	19.63	b* = 30.2
15	19.06	Rice breakfast cereals (T=200°C)
25	16.67	b* = 18.43

4.5. Conclusion

The model system containing native waxy maize starch, glucose, a cocktail of amino acids (glutamic acid, alanine, valine and leucine in the same proportions as the one found in wheat) can be used in further studies on breakfast cereals. Indeed, it mimicked the behaviour of breakfast cereals during the toasting step regarding colour formation and the release of the “volatiles of interest”. This model can therefore be used to study the influence of NaCl on these two markers during the toasting stage of breakfast cereals. Presence of asparagine in the amino-acid cocktail could also allow the study of the influence of NaCl on acrylamide formation. Hence, acrylamide could be used as another marker of the Maillard reactions. The results of such studies are presented in the following chapter (Chapter 5).

5. Influence of NaCl on colour, residual volatiles and acrylamide formation in model systems and breakfast cereals

5.1. Introduction

The model system developed previously mimicked breakfast cereals regarding colour formation and some residual volatile molecules released after the toasting stage. It contained both amino-acids and reducing sugars allowing Maillard reactions to occur. Presence of a large amount of glucose in the model system also allowed caramelisation reactions to happen during the heat treatment. As colour formation and some volatiles released were comparable in model systems and breakfast cereal products, the model was used to observe the influence of NaCl on these parameters, which were markers for the Maillard and/or caramelisation reactions.

pH is a parameter influencing Maillard reactions (section 2.4.2.1). Therefore the pH of the model system was varied to observe whether the influence of NaCl on the several attributes chosen changed depending on the pH of the system.

Asparagine was also added to the amino-acid cocktail of the model system. Such addition allowed the study of the influence of NaCl on acrylamide formation. Acrylamide formation is critical in starchy foods as it was recently discovered in such products and it is a known carcinogenic compound (section 2.4.2.1). When the formulation of a product is modified, the impact of this modification on acrylamide content has to be observed. Indeed, the level of this carcinogenic compound should be kept as low as possible. Based on preliminary observations done before the start of this study, NaCl seemed to impact colour and flavour of a product. Therefore, NaCl might have an influence on Maillard reactions, which could include acrylamide

formation. Hence, acrylamide was added as a marker of the Maillard reactions that should be followed in this study.

5.2. Aims

The aim of this chapter was to observe the influence of NaCl on several attributes: colour, residual volatiles and acrylamide formation at different pHs in the model system previously developed. The NaCl levels, heating times and temperatures were varied. Their impacts on the attributes were followed using several experimental designs.

The conclusions made from the model systems were then verified with some observations made on wet flakes containing different levels of NaCl and toasted for several heating times and temperatures. Observations were also made on final breakfast cereal products.

5.3. System preparation

The model system compounds presented in the following tables were homogeneously mixed according to the procedure presented in section 3.2.1.1. The mixtures were then adjusted to a moisture content of 20 % (wwb) before being toasted. The NaCl concentrations were calculated taking into account that the initial starch moisture content was 10.5 % (wwb).

- Model system at pH 3.5

The model system components are shown in Table 5.1. The pH 3.5 was the pH of the system containing only the compounds presented in Table 5.1. This acidic pH was mainly due to the presence of glutamic acid. Aliquots of 12 g were heated for different periods of time (0 to 25 min) at 230 °C, according to the experimental design presented in Table 5.2.

Table 5.1: Model system composition (pH 3.5).

NaCl concentration (% dwb)	0.00	1.35	2.72	4.09	5.44
Native maize starch (g wet weight)	49.134	48.509	71.824	47.259	46.634
Leucine (g)	0.147	0.147	0.221	0.147	0.147
Valine (g)	0.054	0.054	0.082	0.054	0.054
Alanine (g)	0.032	0.032	0.048	0.032	0.032
Glutamic acid (g)	0.516	0.516	0.774	0.516	0.516
D-Glucose (g)	0.983	0.983	1.475	0.983	0.983
Distilled water (g)	49.134	49.134	49.134	49.134	49.134
NaCl (g)	0.000	0.625	1.879	1.875	2.500
Total	100.000	100.000	100.000	100.000	100.000

Table 5.2: Experimental design used to study the influence of NaCl on colour and volatile development in model systems at pH 3.5.

Sample number	NaCl concentration (% dwb)	Heating time (min)
1	5.44	12.50
2	1.35	6.25
3	0.00	25.00
4	5.44	25.00
5	4.09	6.25
6	5.44	0.00
7	5.44	0.00
8	2.72	12.50
9	0.00	0.00
10	5.44	25.00
11	2.72	0.00
12	2.72	12.50
13	0.00	8.33
14	0.00	16.67
15	1.35	18.75
16	4.09	18.75
17	2.72	12.50
18	0.00	25.00
19	2.72	25.00
20	2.72	12.50

- Model system with pHs between 4.5 and 6.5

The same model composition as that presented in Table 5.1 was used to make models with a pH adjusted to 4.5, 5.5 or 6.5. Adjustment of the model system pH was performed following the procedure presented in section 3.2.1.1. The same experimental design as the one used for the models at pH 3.5 was utilised (Table 5.2). One design was made for each of the pH: 4.5, 5.5, and 6.5. The combination of the 3 designs was then statistically analysed.

- Model system at pH 4.5 containing asparagine

The model systems components are shown in Table 5.3. These compounds were mixed and the pH was adjusted to 4.5. The moisture was then adjusted to 20 % (wwb). The NaCl concentrations (dwb) were calculated taking into account that the initial starch moisture content was 10.5 % (wwb).

Table 5.3: Model system composition.

NaCl concentration (% dwb)	0.00	1.35	2.72	5.44
Native maize starch (g wet weight)	49.137	48.512	47.887	46.637
Leucine (g)	0.134	0.134	0.134	0.134
Asparagine (g)	0.068	0.068	0.068	0.068
Valine (g)	0.048	0.048	0.048	0.048
Alanine (g)	0.029	0.029	0.029	0.029
Glutamic acid (g)	0.467	0.467	0.467	0.467
D-Glucose (g)	0.983	0.983	0.983	0.983
Distilled water (g)	99.631	99.631	99.631	99.631
NaCl (g)	0.000	0.625	1.250	2.500
Total	150.000	150.000	150.000	150.000

The amount of amino-acids in the cocktail (including asparagine) was proportional to the amino acid amount found in wheat, according to Acquistucci et al. (1995). Aliquots of 12 g were heated for different periods of time (6.25 to 25 min) and temperatures (180 °C to 230 °C), according to the experimental design presented in Table 5.4.

Table 5.4: Experimental design used to study the influence of NaCl on colour, on the release of the “volatiles of interest” and on acrylamide formation in model systems.

Sample number	NaCl concentration (% dwb)	Heating time (min)	Temperature (°C)
1	5.44	25.00	180
2	5.44	25.00	180
3	2.72	0.00	180
4	0.00	25.00	180
5	5.44	25.00	230
6	0.00	25.00	230
7	5.44	0.00	230
8	0.00	12.50	205
9	2.72	12.50	205
10	5.44	25.00	230
11	0.00	12.50	180
12	2.72	12.50	205
13	5.44	0.00	180
14	5.44	0.00	230
15	0.00	25.00	230
16	0.00	0.00	205
17	0.00	0.00	230
18	2.72	25.00	205
19	0.00	0.00	180
20	2.72	12.50	230
21	0.00	6.25	180
22	2.72	6.25	180
23	1.35	6.25	205
24	0.00	6.25	230
25	2.72	6.25	230
26	2.72	12.50	205
27	0.00	16.60	180
28	5.44	25.00	196
29	5.44	12.50	180
30	2.72	25.00	180
31	5.44	12.50	180

Wheat, wheat and rice mixture, corn and rice breakfast cereals containing different levels of NaCl were also used in this study. Their preparation was explained in section 3.2.1.5.

5.4. Results and discussion

5.4.1. Influence of NaCl on colour and residual volatile release in model systems at pH 3.5

5.4.1.1. Influence of NaCl on colour formation

Model systems containing between 0 and 5.44 % of NaCl (dwb) were heated in an oven between 0 and 25 min at 230 °C. A picture of the samples obtained is presented in Figure 5.1.

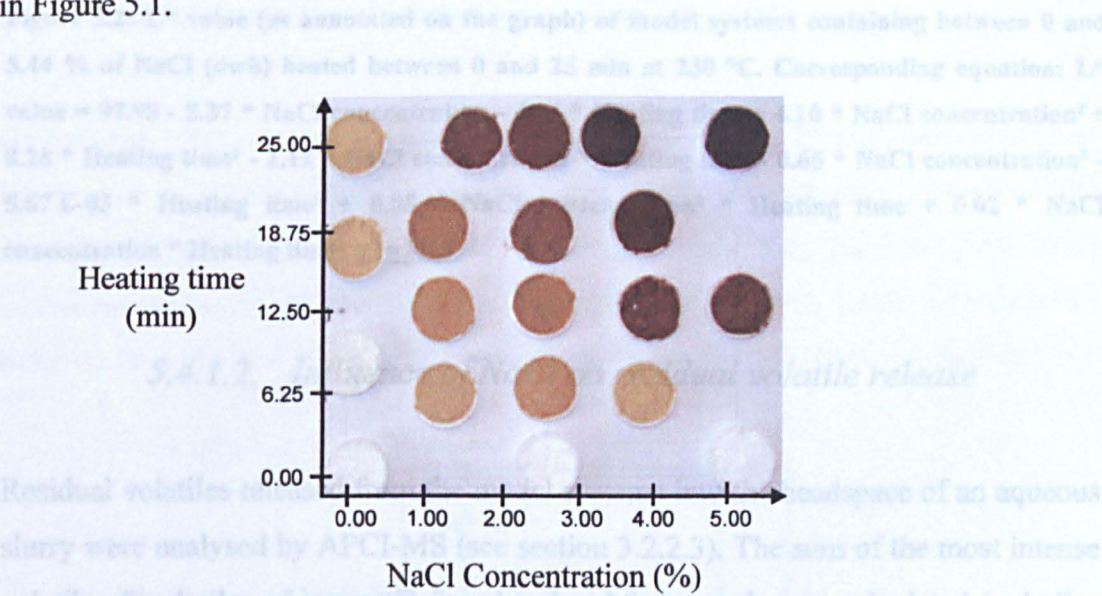


Figure 5.1: Picture of the model systems at pH 3.5 heated at 230 °C versus heating time and NaCl concentration.

Colour measurements were performed on each sample after the heat treatment according to the method presented in section 3.2.2.2. Statistical differences were then calculated with these data (Figure 5.2). Influence of NaCl concentration on the L* value (darkness) of the model systems was significant ($p < 0.05$). The higher the NaCl concentration, the darker the product became after a heat treatment at 230 °C. No trend could be observed concerning the a* and b* values.

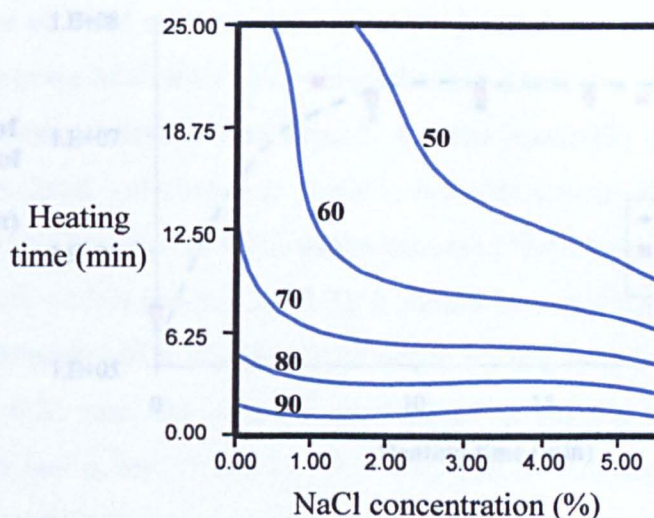


Figure 5.2: L^* value (as annotated on the graph) of model systems containing between 0 and 5.44 % of NaCl (dwb) heated between 0 and 25 min at 230 °C. Corresponding equation: L^* value = $97.98 - 5.37 * \text{NaCl concentration} - 4.94 * \text{Heating time} + 4.10 * \text{NaCl concentration}^2 + 0.28 * \text{Heating time}^2 - 1.12 * \text{NaCl concentration} * \text{Heating time} - 0.65 * \text{NaCl concentration}^3 - 5.67 \text{ E-}03 * \text{Heating time}^3 + 0.08 * \text{NaCl concentration}^2 * \text{Heating time} + 0.02 * \text{NaCl concentration} * \text{Heating time}^2$. $r^2 = 0.98$.

5.4.1.2. Influence of NaCl on residual volatile release

Residual volatiles released from the model systems into the headspace of an aqueous slurry were analysed by APCI-MS (see section 3.2.2.3). The sum of the most intense volatiles (“volatiles of interest”) found in breakfast cereals was calculated (volatiles having a molecular weight of 44, 58, 68, 72, 86 and 102 g/mol, derived from the protonated molecular ion data from APCI-MS). Statistical analysis was then performed on this sum. Results are presented in Figure 5.3 and Figure 5.4.

The trend concerning volatile release can be divided in two distinct parts. The first part concerned the model systems heated between 0 and 6.25 min at 230 °C. During that period, heating time had a significant influence on the intensity of the “volatiles of interest” ($p < 0.05$). They increased when the heating time increased, as shown in Figure 5.3. NaCl concentration did not have any significant influence during that period. The amount of volatiles increased 100 fold during the first minutes of heating. This was most likely due to the Maillard reactions, which were favoured by the high temperatures and led to consequent volatile formation.

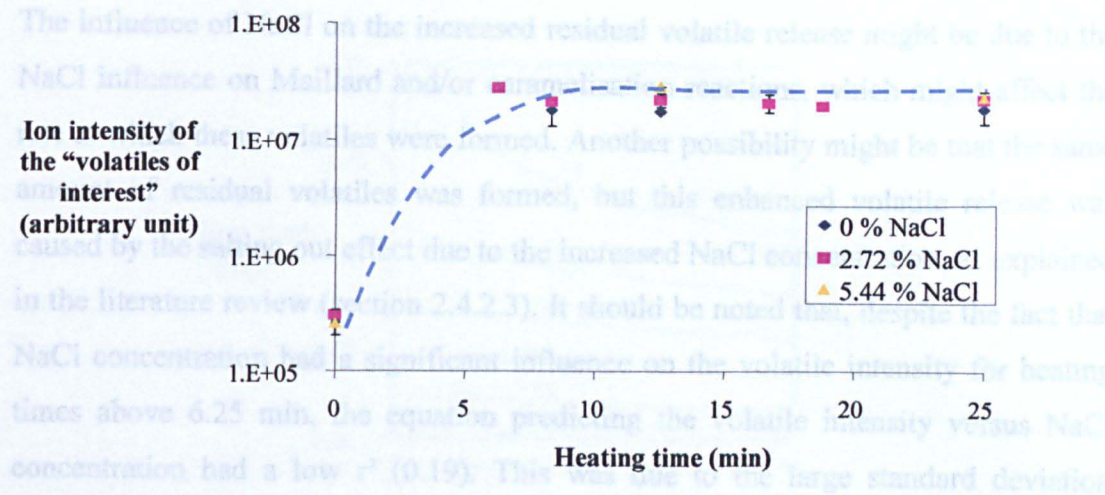


Figure 5.3: Ion intensity of the residual “volatiles of interest” of model systems containing NaCl (concentration between 0 and 5.44 % dwb) versus heating time at 230 °C.

By contrast, when the systems were heated between 6.25 and 25 min, heating time no longer had a significant influence, only the NaCl concentration influenced the intensity of the “volatiles of interest” ($p<0.05$) (Figure 5.4). Increasing the NaCl concentration increased the volatiles intensity. The volatiles taken separately all behaved the same way, following the trend shown for the sum of these volatiles.

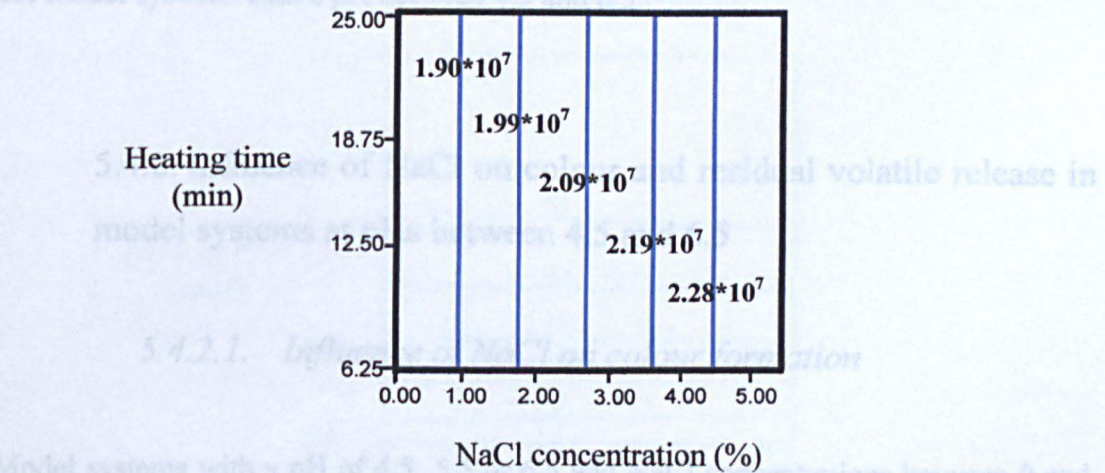


Figure 5.4: Ion intensity of the “volatiles of interest” (as annotated on the graph – arbitrary unit) of model systems containing NaCl (concentration between 0 and 5.44 % dwb) versus heating time (between 6.25 and 25 min) at 230 °C. The data for a heating time of 0 min were removed from this statistical analysis. Intensity of “volatiles of interest” = $1.80 + 1.32 \text{ E}+06 \cdot \text{NaCl concentration}$. $r^2 = 0.19$.

The influence of NaCl on the increased residual volatile release might be due to the NaCl influence on Maillard and/or caramelisation reactions, which might affect the rate at which these volatiles were formed. Another possibility might be that the same amount of residual volatiles was formed, but this enhanced volatile release was caused by the salting out effect due to the increased NaCl concentration, as explained in the literature review (section 2.4.2.3). It should be noted that, despite the fact that NaCl concentration had a significant influence on the volatile intensity for heating times above 6.25 min, the equation predicting the volatile intensity versus NaCl concentration had a low r^2 (0.19). This was due to the large standard deviation between the repetitions.

The model systems used during this study had a pH of 3.5. However, when 1.00 g of breakfast cereal was mixed with 9.00 g of distilled water and the pH was measured after stirring the mixture for 30 min, values between 5.2 and 6.3 were found depending on the product. It was previously presented in the literature review that pH can influence Maillard and caramelisation rates and pathways. Hence, one could wonder if the influence of NaCl on colour and residual volatiles would be influenced by the pH of the model system. The following section presents the results obtained for model systems with a pH between 4.5 and 6.5.

5.4.2. Influence of NaCl on colour and residual volatile release in model systems at pHs between 4.5 and 6.5

5.4.2.1. Influence of NaCl on colour formation

Model systems with a pH of 4.5, 5.5 or 6.5 and NaCl concentrations between 0 and 5.44 % (dwb) were heated for up to 25 min at 230 °C. Colour of the model systems was measured and statistical analyses were performed on the data obtained. Results are presented in Figure 5.5. NaCl concentration, pH and heating time all had a significant influence on the L^* values of the model systems ($p < 0.05$). When NaCl concentration increased, the L^* values decreased regardless of the pH or the heating times. These results follow the same trend compared to the model with a pH of 3.5.

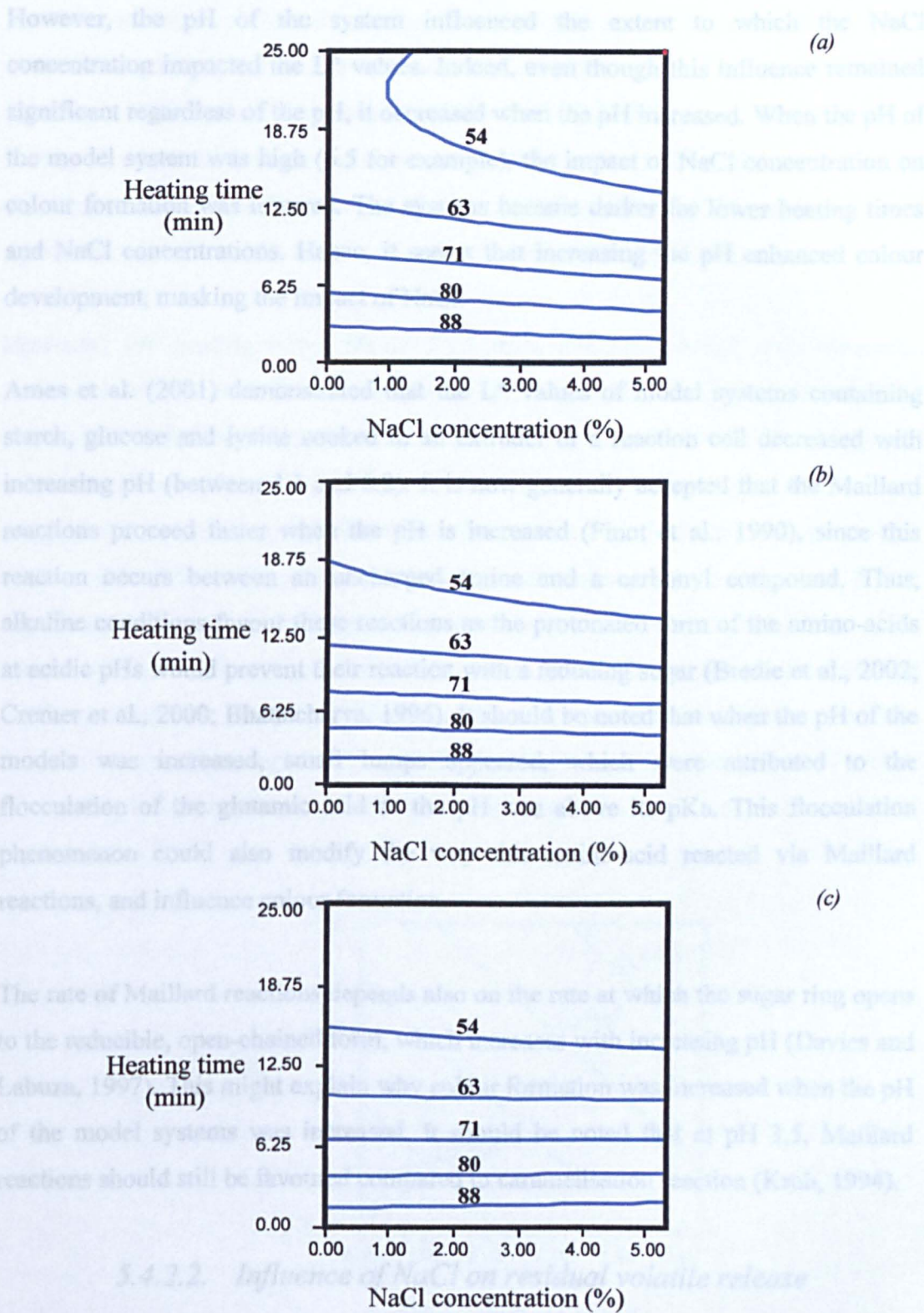


Figure 5.5 : L* values (as annotated on the graph) of model systems containing between 0 and 5.44 % of NaCl (dwb) heated between 0 and 25 min at 230 °C. Models at pH 4.5 (a), 5.5 (b), and 6.5 (c). Corresponding equation: L^* value = $111.12 - 3.58 * \text{NaCl concentration} - 3.99 * \text{Heating time} - 2.60 * \text{pH} + 0.09 * \text{Heating time}^2 - 0.07 * \text{NaCl concentration} * \text{Heating time} + 0.63 * \text{NaCl concentration} * \text{pH}$. $r^2 = 0.97$.

However, the pH of the system influenced the extent to which the NaCl concentration impacted the L^* values. Indeed, even though this influence remained significant regardless of the pH, it decreased when the pH increased. When the pH of the model system was high (6.5 for example), the impact of NaCl concentration on colour formation was lowered. The systems became darker for lower heating times and NaCl concentrations. Hence, it seems that increasing the pH enhanced colour development, masking the impact of NaCl.

Ames et al. (2001) demonstrated that the L^* values of model systems containing starch, glucose and lysine cooked in an extruder or a reaction cell decreased with increasing pH (between 3.1 and 6.8). It is now generally accepted that the Maillard reactions proceed faster when the pH is increased (Finot et al., 1990), since this reaction occurs between an uncharged amine and a carbonyl compound. Thus, alkaline conditions favour these reactions as the protonated form of the amino-acids at acidic pHs would prevent their reaction with a reducing sugar (Bredie et al., 2002; Cremer et al., 2000; Bhattacharya, 1996). It should be noted that when the pH of the models was increased, small lumps appeared, which were attributed to the flocculation of the glutamic acid as the pH was above its pK_a . This flocculation phenomenon could also modify the way this amino-acid reacted via Maillard reactions, and influence colour formation.

The rate of Maillard reactions depends also on the rate at which the sugar ring opens to the reducible, open-chained form, which increases with increasing pH (Davies and Labuza, 1997). This might explain why colour formation was increased when the pH of the model systems was increased. It should be noted that at pH 3.5, Maillard reactions should still be favoured compared to caramelisation reaction (Kroh, 1994).

5.4.2.2. Influence of NaCl on residual volatile release

The sum of the “volatiles of interest” was measured after the model systems with different pHs and NaCl concentrations were heated at 230 °C between 0 and 25 min. As for the models with a pH of 3.5, volatile release can be divided in two distinct parts.

For the model systems heated between 0 and 6.25 min at 230 °C, similar trends were observed regardless of the pH of the model system. Only heating time had a significant influence on the intensity of the “volatiles of interest” ($p<0.05$). They increased when the heating time increased. NaCl concentration did not have any significant influence during that period of time (data not shown). These results are in agreement with the results obtained for the models with a pH of 3.5.

However, for heating times above 6.25 min, different results were observed compared to the models with a pH of 3.5 regarding the influence of NaCl concentration. Indeed, when the models had a pH of 3.5, the “volatiles of interest” release increased when the NaCl concentration increased. Heating time did not have a significant influence on volatile release during that period of time. For the models with a pH of 4.5 or above, both NaCl concentration and heating time had a significant influence ($p<0.05$) on the systems heated for more than 6.25 min. The release of the “volatiles of interest” decreased as the heating time increased and the NaCl concentration increased (Figure 5.6). All the volatiles taken separately followed this trend, except for the volatile that had a molecular weight of 44 g/mol, for which the intensity increased when heating time and NaCl concentration increased.

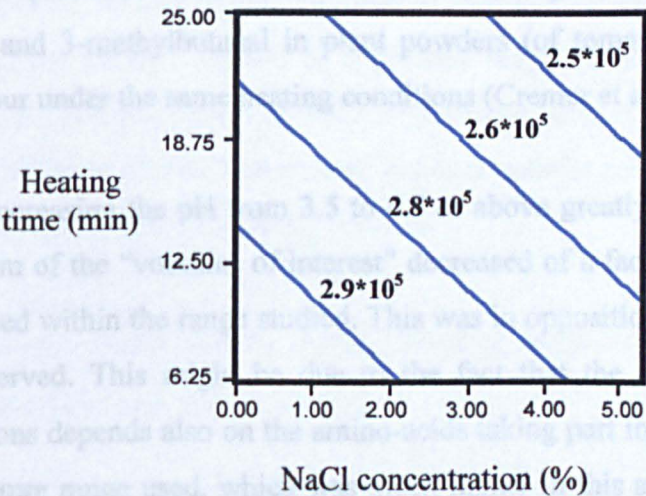


Figure 5.6: Ion intensity of the “volatiles of interest” (as annotated on the graph – arbitrary unit) of model systems containing NaCl (concentration between 0 and 5.44 % dwb) versus heating time (between 6.25 and 25 min) at 230 °C. The data for a heating time of 0 min were removed from this statistical analysis. Intensity of “volatiles of interest” = $3.23 \text{ E}+05 - 14161 * \text{NaCl concentration} - 1826 * \text{Heating time}$, $r^2 = 0.45$.

The pH in itself (between 4.5 and 6.5) did not have a significant influence on the residual volatile release. The reversed trend concerning the influence of NaCl on volatiles at higher pH seemed to indicate that NaCl influenced the generation of volatile molecules. The salting out effect was not the cause for an increased volatile release for the models at pH 3.5. Indeed, the models with a pH between 4.5 and 6.5 had the same NaCl concentration as the models with a pH of 3.5. Hence, the salting-out effect should be similar in all systems regardless of the pH.

As for the models with a pH of 3.5, it should be noted that the r^2 of the equation linking NaCl concentration, heating time and residual volatile release was low, despite the fact that both these factors were significant.

Literature shows that pH exerts a crucial effect on the Maillard reactions when the Amadori compound has been formed (Figure 2.12). At that point, pH affects the pathway taken during the enolisation step. Many studies demonstrated that pH greatly affects the nature and the amount of volatiles formed via Maillard reactions (O'Brien et al., 1998; Bates et al., 1994). The formation of 3-methylbutanal in a low moisture model system composed of glucose and leucine showed a linear pH dependence when heated at 90 °C for 60 min. The higher the pH, the more Strecker aldehydes were produced. The formation of acetaldehyde, 2-methylpropanal, 2-methylbutanal and 3-methylbutanal in plant powders (of tomato and paprika) had similar behaviour under the same heating conditions (Cremer et al., 2000).

In this study, increasing the pH from 3.5 to 4.5 or above greatly influenced volatile release. The sum of the “volatiles of interest” decreased of a factor of 100 when the pH was increased within the range studied. This was in opposition to what Cremer et al. (2000) observed. This might be due to the fact that the influence of pH on Maillard reactions depends also on the amino-acids taking part in the reaction and/or on the temperature range used, which was much higher in this study. The influence of NaCl was different when the models had a pH of 3.5 or 4.5 and above. In the first case, NaCl concentration increased the intensity of residual volatile; but when the pH was increased between 4.5 and 6.5, the NaCl concentration decreased the amount of residual volatile released. These opposite results might be due to the fact that the pH orientated the Maillard reactions pathways differently, as explained above, leading to

a different behaviour of NaCl towards the molecules formed, and changing its influence on the residual volatile release.

Acrylamide is also a by-product of Maillard reactions. The influence of NaCl on its formation in model systems is presented in the following section.

5.4.3. Influence of NaCl on colour, residual volatile release and acrylamide formation in model systems containing asparagine

In order to observe the influence of NaCl on acrylamide formation, the amino-acid asparagine was added to the amino-acid cocktail used in the model system. The pH of the model system was adjusted to 4.5. Indeed, it was observed that the influence of NaCl on volatile release was dependent on the pH when the systems were heated for more than 6.25 min. Acrylamide formation could also be influenced by the pH of the system. Hence, it was chosen to raise the pH of the models to 4.5 in order to be closer to the pH of breakfast cereal products. The parameter heating temperature was also studied. Indeed, breakfast cereal products were toasted within the range of temperature 180 – 235 °C (Chapter 2). Only studies at the highest temperature have so far been reported in this thesis. To observe acrylamide formation, as well as colour and residual volatile release, in a broader range of conditions, the model systems containing different levels of NaCl were heated between 180 and 230 °C for up to 25 min. Influence of NaCl on colour, residual volatile release and acrylamide formation are presented in the following sections.

5.4.3.1. Influence of NaCl, heating temperature and heating time on colour formation

The 3 parameters heating time, heating temperature and NaCl concentration all had a significant influence on the L* value of the model systems heated between 180 and 230 °C for up to 25 min ($p < 0.001$). Figure 5.7 and Figure 5.8 show respectively a picture of the model systems after the heat treatment and the statistical analyses of the models' L* values.

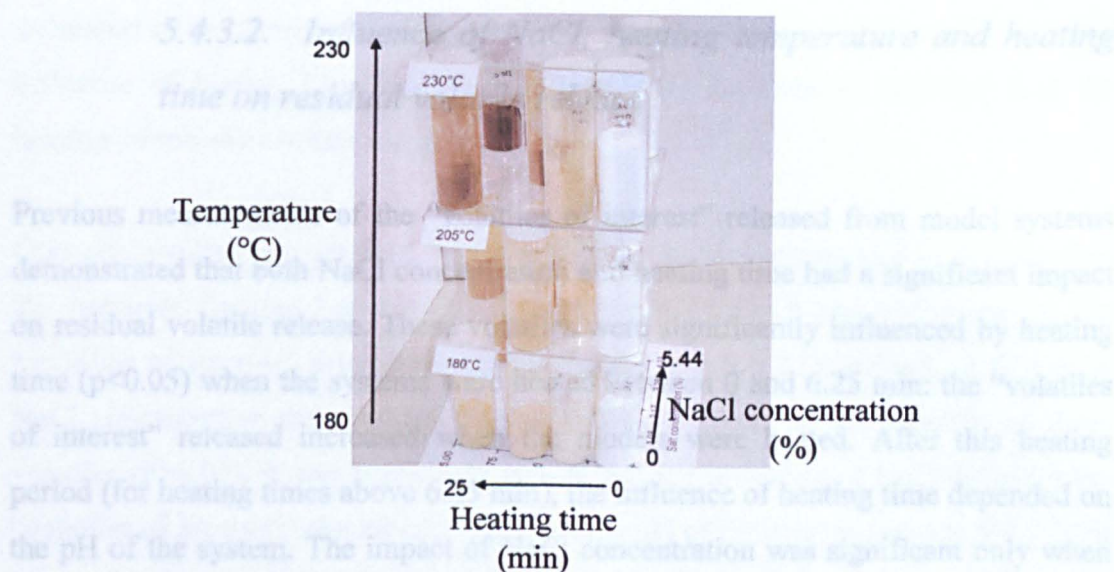


Figure 5.7: Picture of the model systems containing between 0 and 5.44 % of NaCl (dwb) heated between 0 min and 25 min at temperatures between 180 °C and 230 °C.

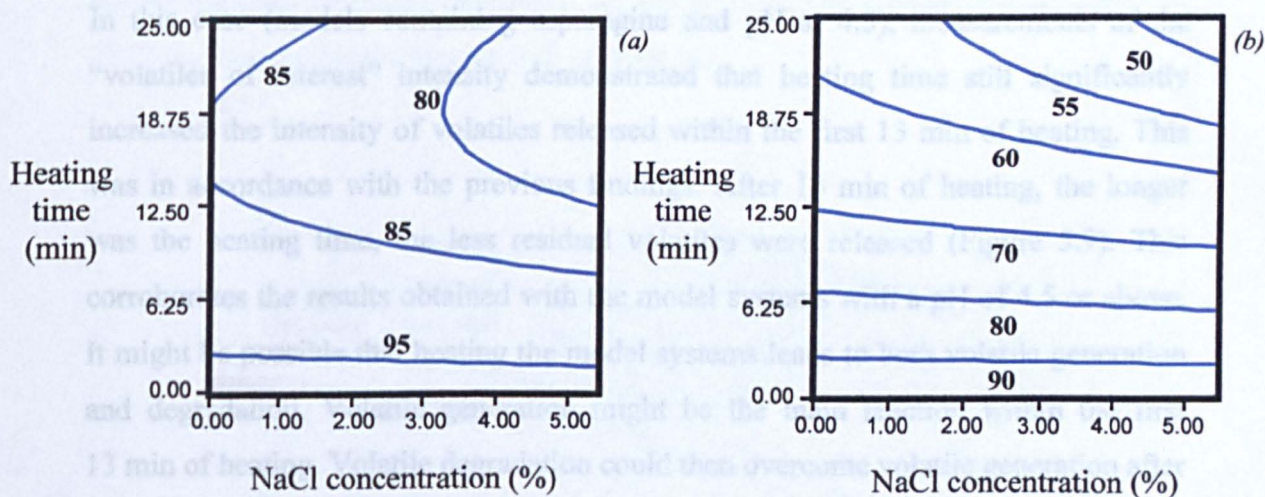


Figure 5.8: L^* value (as annotated on the graph) of model systems (pH 4.5) containing asparagine and between 0 and 5.44 % NaCl (dwb) heated between 0 and 25 min at 180 °C (a) and 230 °C (b). Corresponding equation: $L^* \text{ value} = 105.51 - 0.13 * \text{NaCl concentration} + 2.32 * \text{Heating time} - 0.03 * \text{Temperature} + 0.05 * \text{Heating time}^2 - 0.09 * \text{NaCl concentration} * \text{Heating time} - 0.02 * \text{Heating time} * \text{Temperature}$, $r^2 = 0.98$.

The higher the NaCl concentration, heating temperature and heating time, the darker the products became (the lower the L^* value). a^* or b^* values of the model systems did not follow any trend as a function of the NaCl concentration. These results are in accordance with what was observed previously with all the model systems (pH between 3.5 and 6.5).

5.4.3.2. Influence of NaCl, heating temperature and heating time on residual volatile release

Previous measurements of the “volatiles of interest” released from model systems demonstrated that both NaCl concentration and heating time had a significant impact on residual volatile release. These volatiles were significantly influenced by heating time ($p < 0.05$) when the systems were heated between 0 and 6.25 min: the “volatiles of interest” released increased when the models were heated. After this heating period (for heating times above 6.25 min), the influence of heating time depended on the pH of the system. The impact of NaCl concentration was significant only when the model systems were heated between 6.25 and 25 min and it depended on the pH of the system.

In this case (models containing asparagine and pH at 4.5), measurements of the “volatiles of interest” intensity demonstrated that heating time still significantly increased the intensity of volatiles released within the first 13 min of heating. This was in accordance with the previous findings. After 13 min of heating, the longer was the heating time, the less residual volatiles were released (Figure 5.9). This corroborates the results obtained with the model systems with a pH of 4.5 or above. It might be possible that heating the model systems leads to both volatile generation and degradation. Volatile generation might be the main reaction within the first 13 min of heating. Volatile degradation could then overcome volatile generation after this period of time, explaining the decrease of the “volatiles of interest” intensity.

Heating temperature also influenced the “volatiles of interest” intensity. Increasing heating temperatures from 180 °C to 230 °C decreased the amount of “volatiles of interest” measured in the systems. Increasing heating temperatures might promote volatile molecule break down over formation. However, a study reported that the total yield of 54 residual volatile compounds, identified in glucose - glycine - starch extrudates, increased with temperature within the range 120 °C and 180 °C (Ames et al., 2001). These opposite results might be due to the lower temperatures used or to the fact that a broader range of volatiles was taken into account. Indeed, Bredie et al. (2002) showed that the release of aldehyde molecules from wheat based extrudates

decreased as the temperature increased for a range between 120 °C and 150 °C. The influence of heating time had more influence on the residual volatiles than the heating temperature in this study.

Opposite results were obtained with the models containing asparagine, compared to the previous findings, concerning the influence of NaCl concentrations on the “volatiles of interest” intensity (sections 5.4.1.2 and 5.4.2.2). Indeed, in this case, NaCl concentration does not have any significant impact on the intensity of these volatiles ($p>0.05$). It was previously shown that the influence of NaCl level on the “volatiles of interest” intensity depended on the pH of the system (section 5.4.2.2). It might also depend on the amino-acid cocktail used. Indeed, changing the amino-acid cocktail greatly influenced the residual volatiles obtained, as observed in Chapter 4 during the model system development. Hence, if different pathways were taken during Maillard reactions depending on the amino-acids present, the influence of NaCl on these pathways may as well be different.

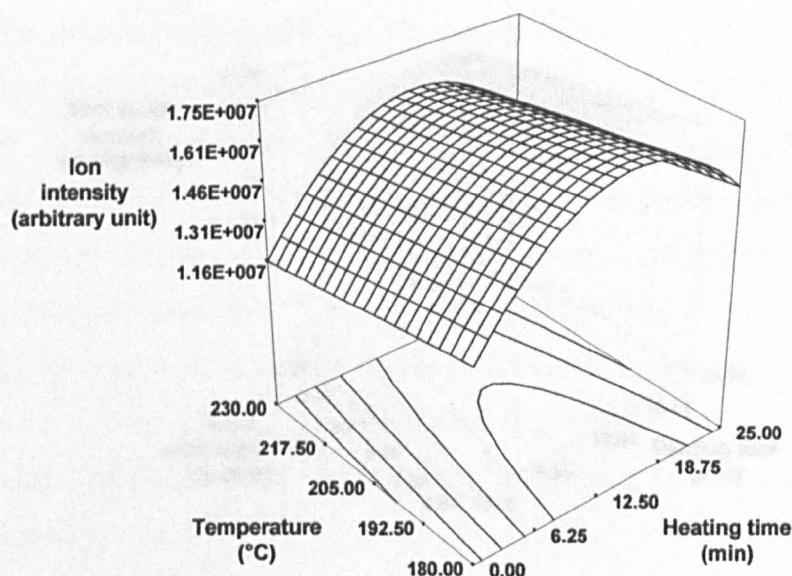


Figure 5.9: Intensity of the “volatiles of interest” released from model systems versus heating times between 0 and 25 min and temperatures between 180 °C and 230 °C. Corresponding equation: $\text{Residual volatiles} = 1.87 \text{ E}+07 + 6.06 \text{ E}+05 * \text{Heating time} - 30714 * \text{Temperature} - 20985 * \text{Heating time}^2$, $r^2 = 0.64$.

5.4.3.3. Influence of NaCl, heating temperature and heating time on acrylamide formation

Acrylamide content was measured for each model system. For the range of experimental conditions investigated, NaCl concentration, heating time and the interaction heating time*heating temperature had a significant influence on acrylamide formation ($p < 0.05$). However, for the temperatures studied, the variable temperature on its own did not significantly influence acrylamide formation (Figure 5.10). According to these model systems, the more NaCl added in the composition, the less acrylamide was formed, regardless of heating time and temperature within the range studied. Influence of NaCl on acrylamide content in different systems has been previously reported. Several authors found that presence of NaCl decreased acrylamide formation in models and food products (Mestdagh et al., 2008a; Pedreschi et al., 2007; Kolek et al., 2006; Levine and Smith, 2005).

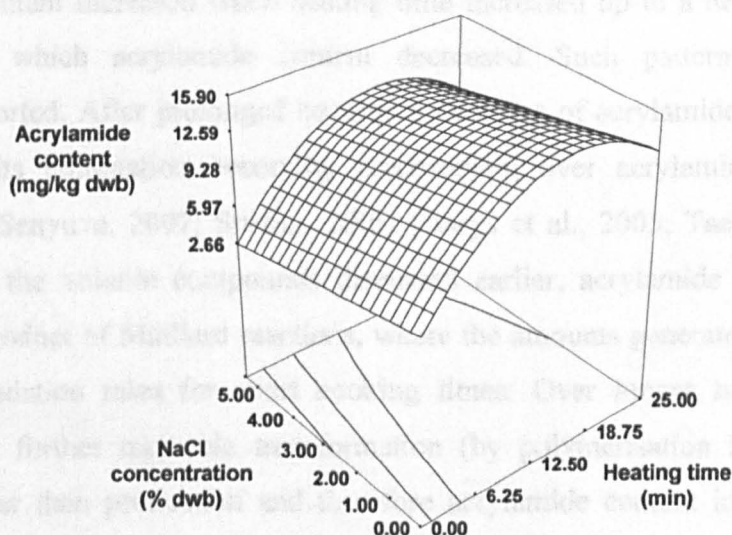


Figure 5.10: Acrylamide formation in model systems heated between 0 and 25 min at a temperature of 205 °C and containing between 0 % and 5.44 % of NaCl (dwb). Corresponding equation: $\text{Acrylamide content} = -380.01 - 0.91 * \text{NaCl concentration} + 3.05 * \text{Heating time} + 3.62 * \text{Temperature} - 0.04 * \text{Heating time}^2 - 8.46 \text{ E-}03 * \text{Temperature}^2 - 9.14 \text{ E-}03 * \text{Heating time} * \text{Temperature}$, $r^2 = 0.74$.

More generally, cations such as Na^+ , K^+ and Ca^{2+} were found to prevent acrylamide formation (Gokmen and Senyuva, 2007; Graf et al., 2006; Levine and Smith, 2005).

Gokmen and Senyuva (2007) reported that acrylamide inhibition by the presence of cations depended on their charge. Added divalent cations seemed to prevent completely acrylamide formation in a model system made of fructose and asparagine, whereas monovalent cations halved the acrylamide formed in the model system. Presence of cations in the reaction mixture limited the Schiff base formation, and thus acrylamide during heating (Gokmen and Senyuva, 2007). Other studies demonstrated that NaCl had catalytic effects on acrylamide polymerisation and formation of polyacrylamide, which may represent one conceivable pathway of acrylamide elimination in a real food matrix (Kolek et al., 2007). Polymerisation of acrylamide was also accelerated considerably by other inorganic compounds, for example, $K_4[Fe(CN)_6]$ and KIO_3 (Kolek et al., 2007). In the present system, the decrease of acrylamide content was proportional to the amount of NaCl added within the concentrations studied. Other studies demonstrated a limited influence of NaCl on acrylamide level above a certain concentration (Kolek et al., 2006).

Acrylamide content increased when heating time increased up to a heating time of 15 min, after which acrylamide content decreased. Such patterns were also previously reported. After prolonged heating, a decrease of acrylamide content was attributed to its elimination becoming predominant over acrylamide formation (Gokmen and Senyuva, 2007; Stadler, 2005; Claeys et al., 2005; Taeymans et al., 2004). As for the volatile compounds discussed earlier, acrylamide might be an intermediate product of Maillard reactions, where the amounts generated are greater than the degradation rates for short cooking times. Over longer heating times, degradation or further molecule transformation (by polymerisation for example) could be greater than production and therefore acrylamide content in the product might then decrease.

Although the model systems used were previously validated in Chapter 4 regarding colour and residual volatiles, the influence of NaCl concentration on these parameters in the model systems might not be the same as in breakfast cereal products. Moreover, the model system pH and composition modified the influence of NaCl on volatile release. Hence, a comparison between the model systems and the food products is needed. Such comparison is presented in the following section.

5.4.4. Comparison between model systems and breakfast cereals

5.4.4.1. Influence of NaCl on colour formation

Colour of breakfast cereal doughs (wheat and rice mixture, corn and rice) and wheat flakes toasted at 230 °C between 0 and 10 or 15 min showed the same trends as the models. The presence of NaCl in the recipe led to significantly darker products ($p<0.05$) (Figure 5.11).

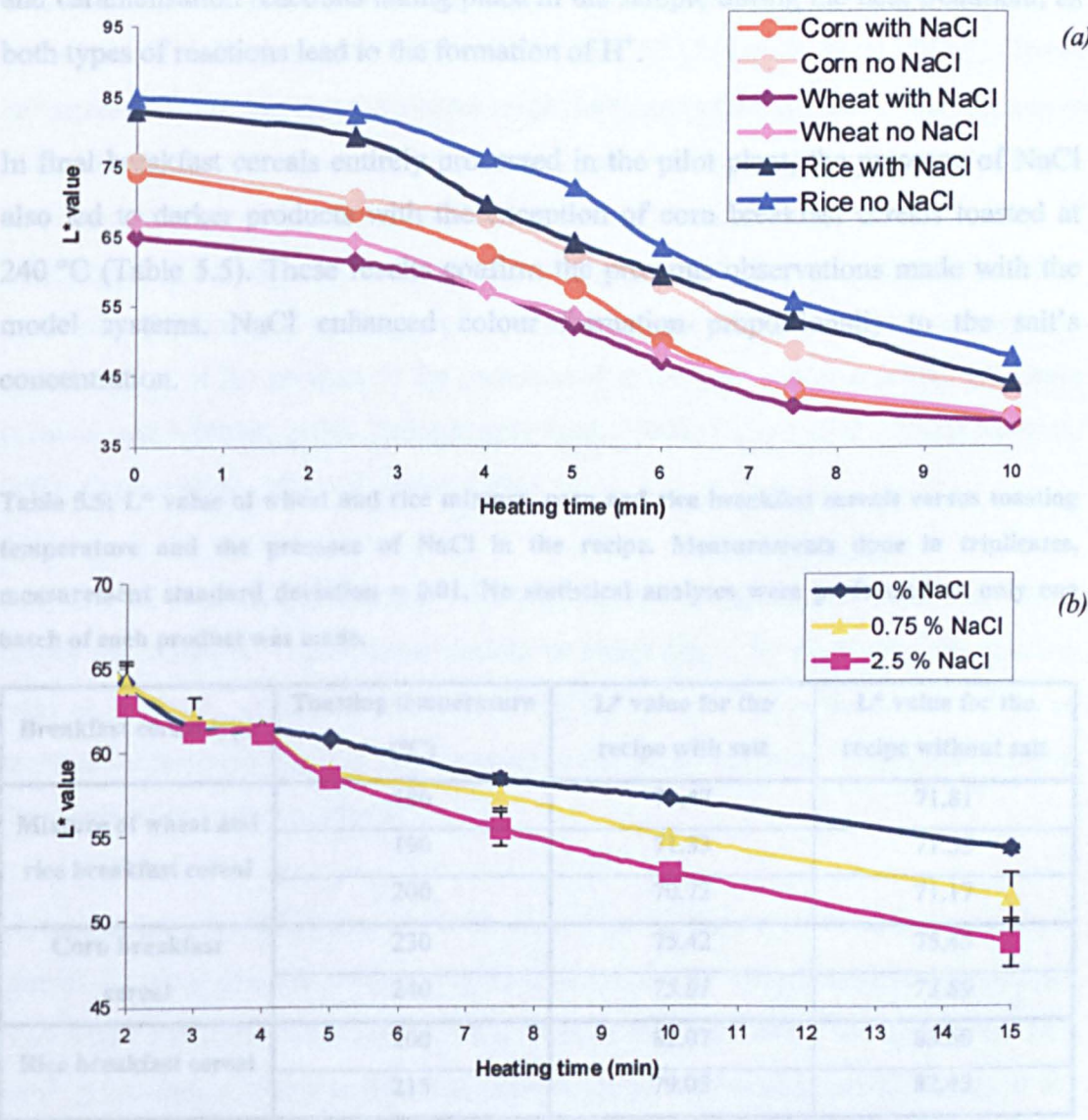


Figure 5.11: L* value of wheat and rice mixture, corn and rice breakfast cereal doughs toasted in an oven (a) and wheat flakes toasted in the small scale toasting device (b), with and without NaCl. They were toasted at 230 °C for different lengths of time. Measurement standard deviation = 0.01, n=3 for the wheat flakes.

A similar pattern was observed for all the samples regardless of the heating time at 230 °C and the type of material used to toast these products when using the oven or the small scale toasting device. The colour enhancement by NaCl seemed to be proportional to the NaCl concentration. The influence of NaCl concentration on colour is more apparent for longer heating times.

The pH of the heated dough or the toasted wet flakes mixed with water in a suspension (1.00 g of dough + 9.00 g of water) decreased as the heating time increased (data not shown). This pH decrease might be the consequence of Maillard and caramelisation reactions taking place in the sample during the heat treatment, as both types of reactions lead to the formation of H⁺.

In final breakfast cereals entirely processed in the pilot plant, the presence of NaCl also led to darker products with the exception of corn breakfast cereals toasted at 240 °C (Table 5.5). These results confirm the previous observations made with the model systems. NaCl enhanced colour formation proportionally to the salt's concentration.

Table 5.5: L* value of wheat and rice mixture, corn and rice breakfast cereals versus toasting temperature and the presence of NaCl in the recipe. Measurements done in triplicates, measurement standard deviation = 0.01. No statistical analyses were performed as only one batch of each product was made.

Breakfast cereal type	Toasting temperature (°C)	L* value for the recipe with salt	L* value for the recipe without salt
Mixture of wheat and rice breakfast cereal	180	71.47	71.81
	190	71.33	71.55
	200	70.72	71.17
Corn breakfast cereal	230	75.42	75.45
	240	75.01	73.89
Rice breakfast cereal	200	82.07	83.60
	215	79.08	82.43

Other authors previously reported that the presence of NaCl in different systems (mainly dry), gave darker products after heating compared to similar products which did not contain NaCl. It was the case for chestnuts during osmotic dehydration

between 25 and 45 °C (Chenlo et al., 2006), wheat grains heated in a steam cooker between 102 and 144 °C (Horrobin et al., 2003), extruded potato starch products (Ferry and Hill, 2007) and bread (Farahnaky and Hill, 2007; Srivastava et al., 1994). However, other systems (mainly liquid) gave opposite results. In model systems containing glucose, glycine and NaCl at various concentrations giving a_w between 0.90 and 0.95, influence of NaCl concentration on the rate of brown colour development was not found to be significant (Kaplow, 1970). In solutions of reducing sugars and amino-acids at pH 6.5, addition of 10 % NaCl (w/w) significantly inhibited colour development (Kwak and Lim, 2004). Soaking potato chips in NaCl was shown to lead to lighter coloured products than those soaked in water and then fried at 120, 140, 160 and 180 °C (Pedreschi et al., 2007). These examples demonstrate that the system might have an impact on how NaCl influences colour formation during a heat treatment. As previously mentioned in the literature review, colour can come from Maillard and/or caramelisation reactions. The rate of both types of reactions and the nature of the products formed are, among other parameters, determined by the reaction conditions, including pH, water activity, the physical state of the product or the presence of metal ions and/or reaction inhibitors (Claude and Ubbink, 2006; Phongkanpai et al., 2006; Robert et al., 2005; Martins, 2003; Cremer et al., 2000; Mottram, 1994).

Presence of NaCl might enhance Maillard and/or caramelisation reactions, hence colour development. This influence might be either direct, by modifying the reaction rates or pathways, or indirect, by influencing the system such as the water activity or moisture content (as NaCl is hygroscopic) or the glass transition temperature (due to the plasticising impact of NaCl).

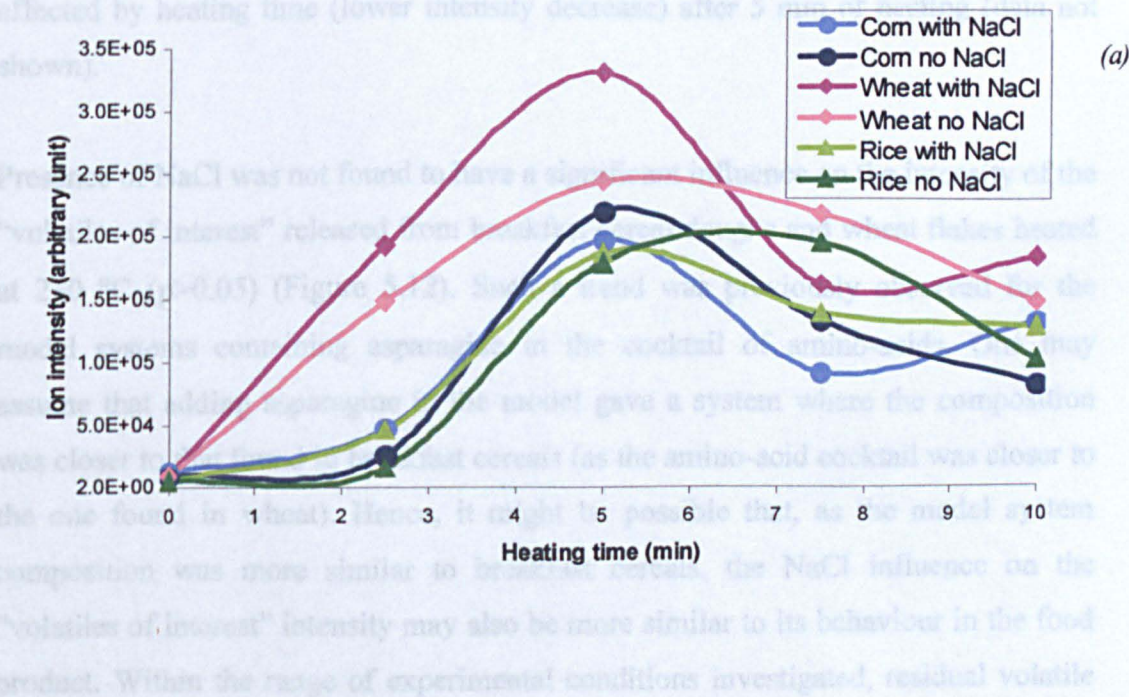
Understanding the influence of NaCl on colour formation in breakfast cereals is crucial as colour is a determinant quality criterion for the consumer. Indeed, too light colour was previously associated with a weak or raw taste and a shorter storage life, while a too dark colour was linked to complaints of over-baked taste (Konings et al., 2007). Understanding the influence of NaCl on the physico-chemistry of the system was the aim of the next chapter (Chapter 6).

5.4.4.2. Influence of NaCl on residual volatile release

Maillard and caramelisation reactions lead to the formation of colour compounds but also volatile molecules. The above results did show that NaCl influenced colour formation in breakfast cereals, which was in accordance with the results of the model systems.

The “volatiles of interest” intensity was also measured in breakfast cereal products with the aim to investigate whether NaCl had an impact on these compounds formations, as it seemed to be indicated by the model systems. Volatiles might take part to the overall aroma of the product. An influence of NaCl on the formation of these molecules might translate a possible impact on the sensory properties of breakfast cereals.

Breakfast cereal doughs were heated at 230 °C between 0 and 10 min in an oven and wet wheat flakes were toasted at 230 °C between 0 and 15 min in the small toasting device. During the first minutes of the heat treatment (between 0 and 5 min), the longer was the heat treatment, the higher was the residual volatile intensity. After 5 min of heating at 230 °C, the volatile intensity decreased as the heating time increased (Figure 5.12).



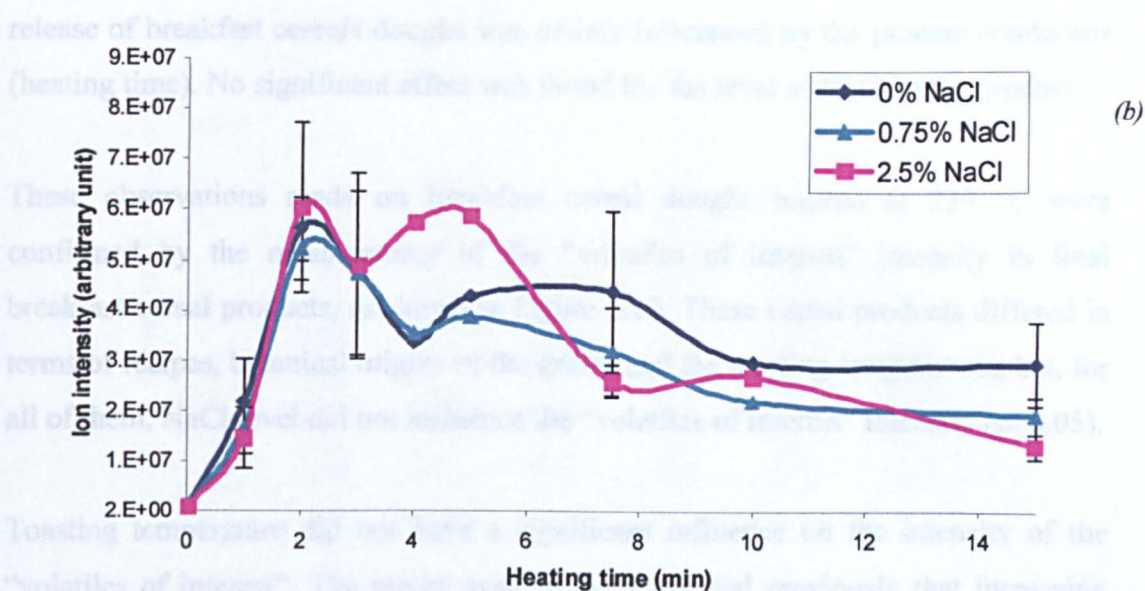


Figure 5.12: Intensity of the “volatiles of interest” released from wheat and rice mixture, corn and rice breakfast cereal doughs (a) and wheat flakes (b), with and without NaCl, versus heating time at 230 °C (error bars not shown in the first graph (a) for clarity purposes, average standard deviation = 1.2 E+04).

Same results were observed for all types of breakfast cereal products. Influence of heating time was similar to the trends observed in model systems with a pH of 4.5 or above (including the model systems containing asparagine). All the “volatiles of interest” followed the same trend: they increased during the first minutes of heating, and then decreased. The volatiles with a molecular weight of 58 g/mol were less affected by heating time (lower intensity decrease) after 5 min of heating (data not shown).

Presence of NaCl was not found to have a significant influence on the intensity of the “volatiles of interest” released from breakfast cereal doughs and wheat flakes heated at 230 °C ($p > 0.05$) (Figure 5.12). Such a trend was previously observed for the model systems containing asparagine in the cocktail of amino-acids. One may assume that adding asparagine in the model gave a system where the composition was closer to that found in breakfast cereals (as the amino-acid cocktail was closer to the one found in wheat). Hence, it might be possible that, as the model system composition was more similar to breakfast cereals, the NaCl influence on the “volatiles of interest” intensity may also be more similar to its behaviour in the food product. Within the range of experimental conditions investigated, residual volatile

release of breakfast cereals doughs was mainly influenced by the process conditions (heating time). No significant effect was found for the level of NaCl in the product.

These observations made on breakfast cereal doughs toasted at 230 °C were confirmed by the measurement of the “volatiles of interest” intensity in final breakfast cereal products, as shown in Figure 5.13. These cereal products differed in terms of recipes, botanical origins of the grains and the toasting temperatures but, for all of them, NaCl level did not influence the “volatiles of interest” intensity ($p>0.05$).

Toasting temperature did not have a significant influence on the intensity of the “volatiles of interest”. The model systems demonstrated previously that increasing the heating temperature decreased the amount of residual volatiles. These differences might be due to the fact that the toasting time of the breakfast cereals (37 s) were shorter than that of the model systems (between 0 and 25 min). Hence, the impact of heating temperature might not be relevant in normal production of cereals. Indeed, the product may not achieve the state of greater degradation of volatile products compared to their generation.

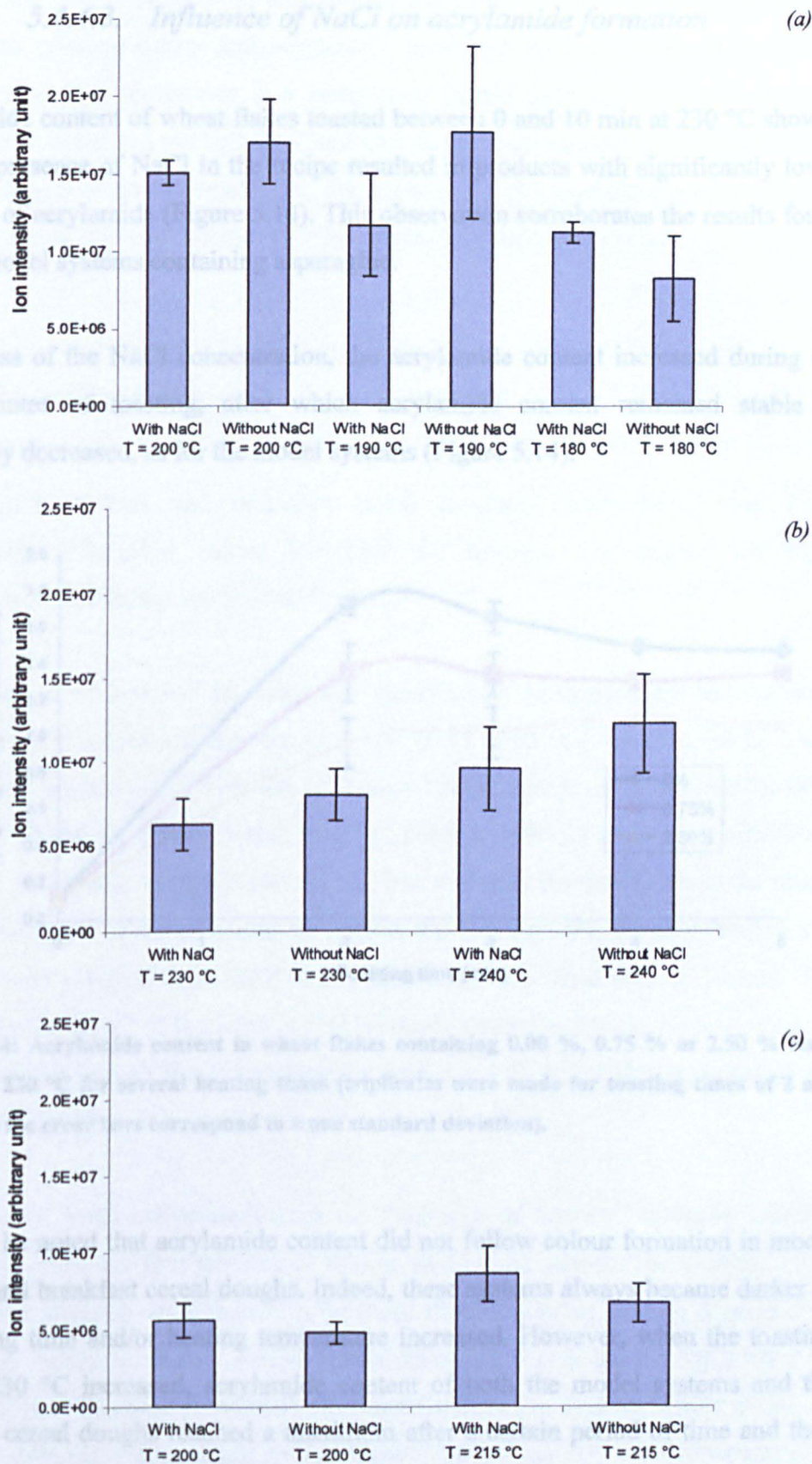


Figure 5.13: Residual volatile intensity of wheat (a), corn (b) and rice (c) breakfast cereals, toasted with and without NaCl. The temperature (T) noted next to the type of breakfast cereal corresponds to the toasting temperature used.

5.4.4.3. Influence of NaCl on acrylamide formation

Acrylamide content of wheat flakes toasted between 0 and 10 min at 230 °C showed that the presence of NaCl in the recipe resulted in products with significantly lower amounts of acrylamide (Figure 5.14). This observation corroborates the results found for the model systems containing asparagine.

Regardless of the NaCl concentration, the acrylamide content increased during the first minutes of toasting, after which acrylamide content remained stable or eventually decreased, as for the model systems (Figure 5.14).

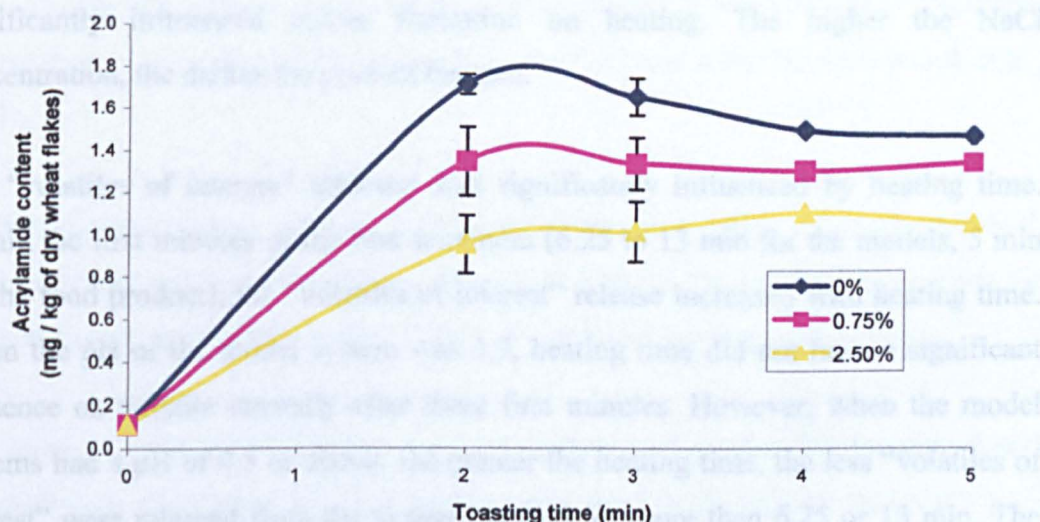


Figure 5.14: Acrylamide content in wheat flakes containing 0.00 %, 0.75 % or 2.50 % NaCl toasted at 230 °C for several heating times (triplicates were made for toasting times of 2 and 3 min, and the error bars correspond to \pm one standard deviation).

It should be noted that acrylamide content did not follow colour formation in model systems and breakfast cereal doughs. Indeed, these systems always became darker as the heating time and/or heating temperature increased. However, when the toasting time at 230 °C increased, acrylamide content of both the model systems and the breakfast cereal doughs reached a maximum after a certain period of time and then decreased. Moreover, within the temperatures studied, the variable temperature on its own did not have a significant impact on acrylamide formation. It was previously reported that the degree of browning was not a good guide to acrylamide level (Sadd

and Hamlet, 2005). Moreover, Rufian-Henares et al. (2006) did not observe any correlation between colour and acrylamide level in breakfast cereals. It should be kept in mind that acrylamide is a compound formed only via Maillard reactions, while colour is formed via both Maillard and caramelisation reactions. Therefore, the different trends observed concerning the impact of NaCl on colour and acrylamide might be due to different impacts of NaCl on each of these reactions.

5.5. Conclusions

Both model systems and breakfast cereal products demonstrated that NaCl significantly influenced colour formation on heating. The higher the NaCl concentration, the darker the product became.

The “volatiles of interest” intensity was significantly influenced by heating time. Within the first minutes of the heat treatment (6.25 to 13 min for the models, 5 min for the food product), the “volatiles of interest” release increased with heating time. When the pH of the model system was 3.5, heating time did not have a significant influence on volatile intensity after these first minutes. However, when the model systems had a pH of 4.5 or above, the greater the heating time, the less “volatiles of interest” were released from the systems heated for more than 6.25 or 13 min. The “volatiles of interest” of breakfast cereal products toasted at 230 °C corroborated the observation made with the model systems with a pH of 4.5 or above regarding the influence of heating time.

The effect of NaCl concentration on the “volatiles of interest” intensity was not significant within the first minutes of heating (6.25 to 13 min for the models, 5 min for the food product). Its influence after these first minutes of heating depended on the system. Indeed, in model systems with a pH of 3.5, increasing the NaCl led to an increase of the “volatiles of interest” intensity. However, in systems with a pH of 4.5 or above without the presence of asparagine, increasing the NaCl concentration decreased the “volatiles of interest” amount. In models with a pH of 4.5 and containing asparagine, NaCl level did not have a significant influence on “volatiles

of interest” intensity. In breakfast cereal products, no influence of NaCl concentration could be observed, which corroborated the results of this last model system. The composition and pH of the models influenced tremendously the impact of NaCl on residual volatiles. However, the closest model system to breakfast cereal products in terms of pH and composition gave similar NaCl impact compared to breakfast cereal products. The absence of significant impact of NaCl level on the 6 residual volatile molecules chosen might indicate that these volatiles may not be key notes in the aroma and flavour of the products as blander tasting products were obtained in the absence of NaCl. Another hypothesis could be that these volatiles participate to the flavour of the product; but even though the NaCl concentration did not affect their generation, decreasing the NaCl level decreased the flavour perception giving a blander taste to the product. Indeed, it was previously found that a perceptual interaction exists between the salty taste and some flavours (Cook et al., 2003).

NaCl concentration also influenced acrylamide formation and/or degradation in both model systems and breakfast cereal products. The higher the NaCl concentration, the less acrylamide was present in the product for identical processing conditions.

The impact of NaCl on Maillard reactions (colour and acrylamide formation) and eventually caramelisation reactions (colour formation) might indicate that NaCl does not give food only its salty taste. It could also influence the generation of aroma and flavour molecules during the toasting step as they are formed via Maillard and caramelisation reactions. It could explain the bland taste obtained for the products without NaCl. If that was the case, sodium reduction in food products without any change in taste might be even more challenging. The understanding of the influence of NaCl on Maillard and caramelisation reactions is therefore necessary to be able to replace this ingredient without altering the product quality.

It was mentioned previously that Maillard reactions can be influenced, among other parameters, by the physical state and the moisture content of the product. As NaCl is a plasticiser, it might impact the T_g of breakfast cereals. In the model system used in this study, NaCl might influence the T_g of the amorphous parts of the starch granules (structure of the starch granule presented in section 2.3.1.1). It was shown that

molecules with a weight below 1000 g/mol can freely penetrate the granules (Hoseney, 1986). Hence, the Maillard reactions occurring between the glucose and the amino-acids in the amorphous parts of the starch granules might be affected by the physical state of the system. This could explain the influence of NaCl on colour and acrylamide formation. Presence of NaCl might also have an influence on the product moisture as this component is hygroscopic. Moisture changes might influence the rates and pathways of the Maillard and/or caramelisation reactions.

The next chapter reports work that aimed to determine whether the influence of NaCl on colour formation (hence Maillard and caramelisation) can be linked to the glass transition temperature of the system or its moisture sorption behaviour.

6. Influence of NaCl on colour formation via Tg and moisture sorption modifications

6.1. Introduction

To decrease the sodium content of cereal based products without changing any of their characteristics, a good understanding of the role of NaCl in such products is key. The main reason sodium chloride is added to cereal based products is for its salty taste, but the taste is not the only parameter NaCl is influencing. The previous chapter demonstrated that it also has an impact on colour formation, on some residual volatile molecules intensity (depending on the conditions) and on acrylamide formation. Colour, volatiles and acrylamide can be formed during Maillard reactions. Caramelisation could also be responsible for some colour and volatile formation.

As presented in the literature review (section 2.4.2.1), Maillard reactions can be influenced by the system properties, such as its moisture content. Influence of the moisture content on Maillard reactions rates was generally attributed to modification of the water availability, which is necessary for the reactants' mobility. Maillard reaction rates can also be influenced by the physical state of a system (glassy versus rubbery) as it changes the diffusion and mobility of the reactants. Mobility and diffusion of the reactants are critical factors for Maillard reactions as they consist at first in a bimolecular condensation step.

NaCl is a hygroscopic compound, meaning that the presence of NaCl in a system favours water sorption. It might be possible that this ability of NaCl to sorb water could also influence the water evaporation from a system during a heat treatment. If the presence of sodium chloride could slow down water evaporation, Maillard

reactions might be favoured as the reactants would be mobile longer thanks to the extended presence of water. Hence, it could explain the influence of this compound on colour and acrylamide formation. Moreover, as sodium chloride has a low molecular weight, it can act as a plasticiser in the starch based system. Therefore, the presence of NaCl in breakfast cereals might influence their glass transition temperatures. NaCl might extend the time the product is in a rubbery state before entering a glassy state at the end of the toasting tunnel, hence allowing Maillard reactions to proceed longer.

As previously mentioned, Maillard reactions are favoured when the product has an intermediate to low moisture content and when it is in a rubbery state. If NaCl is present in a product, the glass transition temperature of the system might be lower than the same product without NaCl, enlarging the window where Maillard reactions are favoured. At the same time, presence of NaCl might slow down the evaporation rate of water as it is a hygroscopic compound. The temperature rise would be slowed as well, favouring Maillard reactions over a longer period of time. This could explain the impact of NaCl on colour and acrylamide formation.

6.2. Aims

The present study now reported in this chapter aimed at investigating the two possible ways NaCl could influence Maillard reactions in cereal based products, as presented in the introduction of this chapter. These two hypotheses are both linked to the potential impact of NaCl on molecular mobility and diffusion. Colour was the Maillard reactions marker chosen for this study to test the two hypotheses. The first hypothesis was the possible correlation between colour formation and the hygroscopic behaviour of a system. The second was the influence of NaCl on the glass transition temperature of the system and a hypothetical correlation between Tg and colour formation.

The two hypotheses were tested using the model system developed earlier, mixed with several types of plasticisers. However, the models used in this chapter contained

pregelatinised starch instead of native starch. Indeed, the impact of NaCl on Tg and the possible repercussions on Maillard reactions (hence on colour development) were based on the fact that breakfast cereal products become glassy at the end of the process. Hence, Maillard reactions in the model systems might be enhanced if the entire model is glassy, instead of partly glassy and partly crystalline as is the case with native starch which had been in the previous model systems.

6.3. System preparation

The model systems were prepared according to the procedure presented in the section 3.2.1.2. The compounds were mixed and the moisture content of each model system was adjusted to 15 % (wwb). The plasticiser concentrations were calculated considering an initial starch moisture content of 10.5 % (wwb).

- Models with pregelatinised starch, glucose, an amino-acid cocktail and NaCl

The model system composition is shown in Table 6.1. Aliquots of 12 g were heated for different periods of time (0 to 10 min) at 230 °C, according to the experimental design presented in Table 6.2.

Table 6.1: Model system composition used to study the influence of NaCl on colour development.

NaCl concentration (% dwb in the model system)	0.00	1.34	2.69	4.02	5.36
Pregelatinised waxy maize starch (g wet weight)	49.134	48.509	47.883	47.259	46.634
Leucine (g)	0.147	0.147	0.147	0.147	0.147
Valine (g)	0.054	0.054	0.054	0.054	0.054
Alanine (g)	0.032	0.032	0.032	0.032	0.032
Glutamic acid (g)	0.516	0.516	0.516	0.516	0.516
D-Glucose (g)	0.983	0.983	0.983	0.983	0.983
Distilled water (g)	≅ 250	≅ 250	≅ 250	≅ 250	≅ 250
NaCl (g)	0.000	0.625	1.879	1.875	2.500

Table 6.2: Experimental design used to study the influence of NaCl and heating time on colour formation of model systems heated at 230 °C.

Run Number	NaCl concentration (% dwb)	Heating time (min)
1	2.68	5.00
2	4.02	2.50
3	0.00	10.00
4	5.36	5.00
5	5.36	0.00
6	5.36	10.00
7	0.00	10.00
8	4.02	7.50
9	5.36	0.00
10	2.68	5.00
11	2.68	10.00
12	0.00	3.33
13	1.34	2.50
14	2.68	0.00
15	1.34	7.50
16	0.00	6.66
17	0.00	0.00
18	5.36	10.00
19	2.68	5.00
20	1.35	10.00
21	4.02	10.00

- Models with pregelatinised starch and NaCl

Model systems composed of pregelatinised starch and NaCl (concentrations used: 0.00, 0.50, 1.00, 2.50 and 5.00 % dwb) were prepared following the same procedure as for the models presented above. They were then stored over P₂O₅ or saturated salt solutions of LiCl, CH₃COOK, K₂CO₃, NaNO₂, NaCl and water which gave water activity values of 0, 0.11, 0.23, 0.43, 0.64, 0.75 and 1.00 respectively (Biliaderis et al., 1999). The detailed procedure was presented in section 3.2.1.6.

- Models with different types of plasticisers

The model system components were weight precisely as indicated in Table 6.3 and the systems were prepared in the same way as the previous models. They contained a range of plasticisers, as shown in Table 6.3. Aliquots of 12 g were heated at 230 °C between 0 and 5 min.

Table 6.3: Model system compositions: NaCl replacement by other plasticisers.

Plasticiser concentration (% dwb)	No plasticiser	Blank without plasticiser	Blank with 5.36 % NaCl	Blank with 5.36 % KCl	Blank with 25.00 % trehalose	5.36 % NaCl	5.36 % KCl	25.00 % trehalose
Pregelatinised waxy maize starch (g)	147.402	149.902	142.851	142.851	108.251	139.902	139.902	105.302
Leucine (g)	0.441	0.000	0.000	0.000	0.000	0.441	0.441	0.441
Valine (g)	0.162	0.000	0.000	0.000	0.000	0.162	0.162	0.162
Alanine (g)	0.096	0.000	0.000	0.000	0.000	0.096	0.096	0.096
Glutamic acid (g)	1.548	0.000	0.000	0.000	0.000	1.548	1.548	1.548
D-Glucose (g)	2.949	0.000	0.000	0.000	0.000	2.949	2.949	2.949
Distilled water (g)	≅ 800	≅ 800	≅ 800	≅ 800	≅ 800	≅ 800	≅ 800	≅ 800
Plasticiser (g)	0.000	0.000	7.500	7.500	34.600	7.500	7.500	34.600

Wheat and rice mixture, corn and rice breakfast cereals containing different levels of NaCl were used in this study. Their preparation was explained in section 3.2.1.5.

6.4. Results and discussion

6.4.1. Influence of NaCl on colour development of model systems

Model systems containing pregelatinised starch, glucose, a cocktail of amino-acids and NaCl between 0 and 5.36 % (dwb) (Table 6.1) with an initial moisture content of 15 % (wwb) were heated in an oven between 0 and 10 min at 230 °C. After the heat treatment, colour measurements were performed on each sample (Figure 6.1) according to the method presented in section 3.2.2.2.

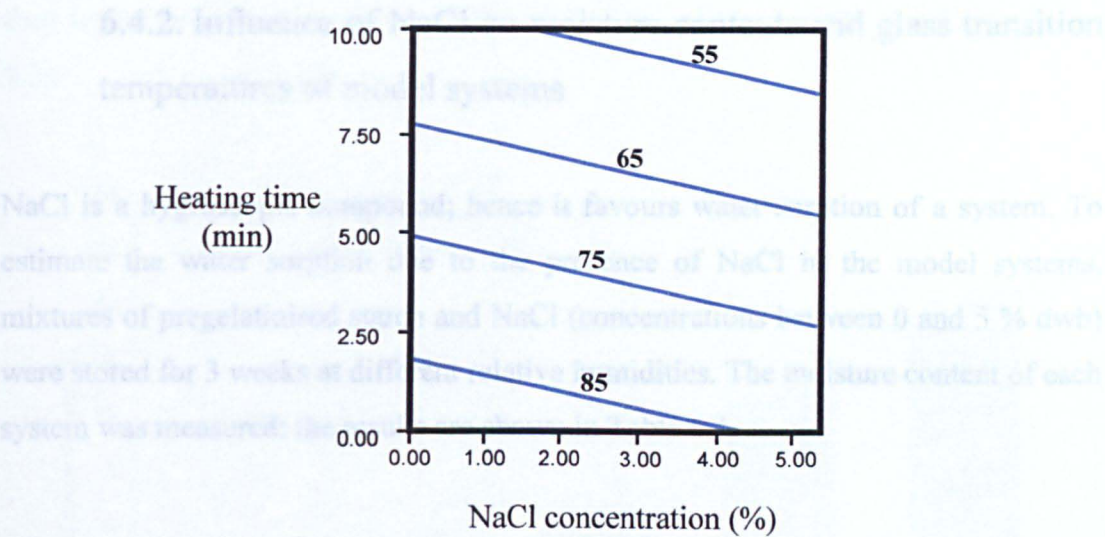


Figure 6.1: L* value (as annotated on the graph) of model systems heated at 230 °C versus heating time (min) and NaCl concentration (% dwb). Corresponding equation: $L^* \text{ value} = 93.23 - 1.50 * \text{NaCl concentration} - 3.61 * \text{Heating time}$, $r^2 = 0.92$.

NaCl concentration and heating time have both a significant influence on the L* value of the model systems ($p < 0.05$). The higher the NaCl concentration and the heating time, the darker the products became (the lower the L* values). These results confirmed the previous findings, showing that the influence of NaCl on colour development followed the same trend regardless of the type of starch used (native or pregelatinised).

It should be noted that pregelatinised starch gave darker products than native starch when heated under the same conditions with the presence of amino-acids and glucose. If NaCl influenced Maillard reactions via modifications of the glass transition temperature, the darker colour obtained with pregelatinised starch might be due to the fact that the entire system was amorphous. Hence, a change in glass transition temperature due to the level of NaCl would have more repercussions on Maillard reactions, hence on colour development, than with systems containing native starch (where crystalline regions are also present).

6.4.2. Influence of NaCl on moisture contents and glass transition temperatures of model systems

NaCl is a hygroscopic compound; hence it favours water sorption of a system. To estimate the water sorption due to the presence of NaCl in the model systems, mixtures of pregelatinised starch and NaCl (concentrations between 0 and 5 % dwb) were stored for 3 weeks at different relative humidities. The moisture content of each system was measured; the results are shown in Table 6.4.

Table 6.4: Average moisture content (n=3) of model systems containing pregelatinised starch and NaCl (0 to 5 % dwb) and stored for 3 weeks at several relative humidities. The letters, by the moisture values, indicate significant differences between the samples for a specific relative humidity ($p < 0.05$).

Relative humidities	100%	75%	43%	23%
0% NaCl	31.97 ^a	15.88 ^b	9.84 ^a	6.87 ^a
0.5% NaCl	31.74 ^a	14.68 ^a	9.96 ^a	6.63 ^a
1% NaCl	32.92 ^{a,b}	15.98 ^b	9.85 ^a	6.79 ^a
2.5% NaCl	33.74 ^b	16.27 ^{b,c}	10.14 ^{a,b}	6.80 ^a
5% NaCl	33.98 ^b	16.59 ^c	10.53 ^b	7.05 ^a

For relative humidities of 43 % or above, presence of NaCl in the system significantly increased its moisture sorption during storage. The more NaCl present, the higher was the moisture content. The moisture content increase due to the presence of 5 % NaCl was up to 2 % (wwb) at a relative humidity of 100 %. Similar trends were observed by other authors. Laaksonen and Roos (2003) observed that wheat doughs prepared with and without NaCl and kept at constant a_w until equilibration did not reach the same moisture content. Steady state water contents of doughs with added NaCl were slightly higher. Martelli et al. (2006) showed that increasing the NaCl concentration in chicken feather keratin films favoured water adsorption by the polymeric network.

Moisture content of the wheat and rice mixture breakfast cereals also demonstrated that presence of NaCl in the recipe led to significantly higher moisture uptake when

they were stored for 3 weeks at relative humidities of 43 %, 64 % and 75 % (Figure 6.2).

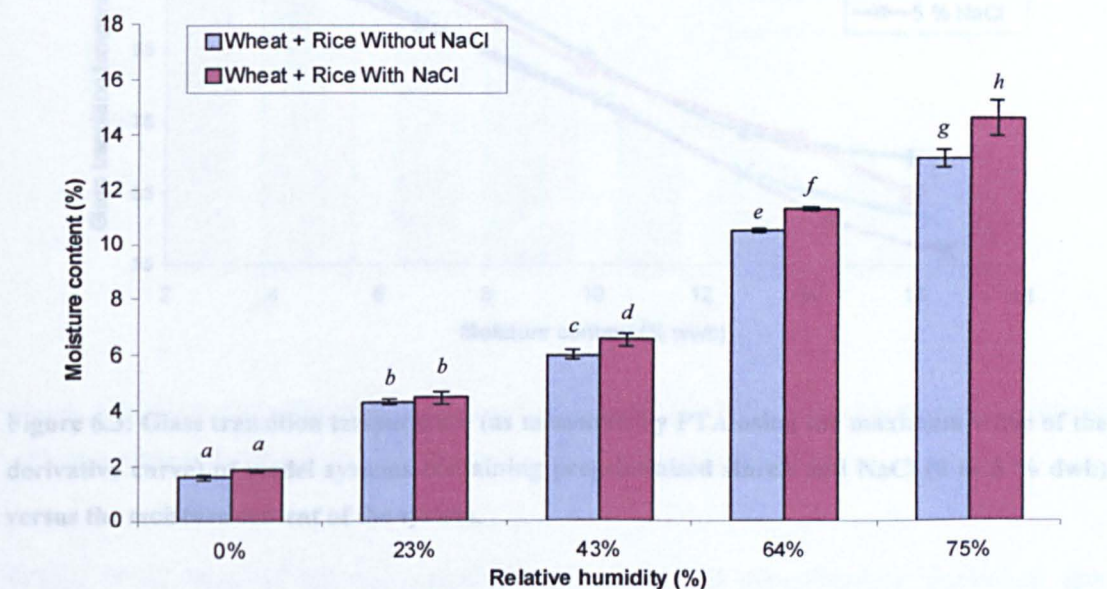


Figure 6.2: Average moisture content ($n=3$) of wheat and rice mixture breakfast cereal products after storage for 3 weeks at different relative humidities (the error bars correspond to \pm one standard deviation). The letters above each bar indicate significant differences between the samples ($p<0.05$).

Presence of NaCl in a system might have a strong influence on the glass transition temperature (T_g) of the product, which might be sufficient to cause a significant change in the physical state of the product while heating. To evaluate the influence of sodium chloride on T_g , the glass transition temperatures of models containing pregelatinised starch and NaCl, and stored for 3 weeks at several relative humidities, were measured according to the method given in section 3.2.2.5 (Figure 6.3).

(Figure 6.4). Similar results were obtained by DSC (data not shown). It should be noted that in Figure 6.4, T_g was plotted versus the relative humidity at which the product was stored and not versus the moisture content of the product. As NaCl is hygroscopic, the moisture content of a product containing NaCl tends to be higher, as previously observed. Water is also a known plasticiser. Hence, the higher moisture content of products containing NaCl can also be responsible, together with NaCl, for the lowering of T_g (Figure 6.4).

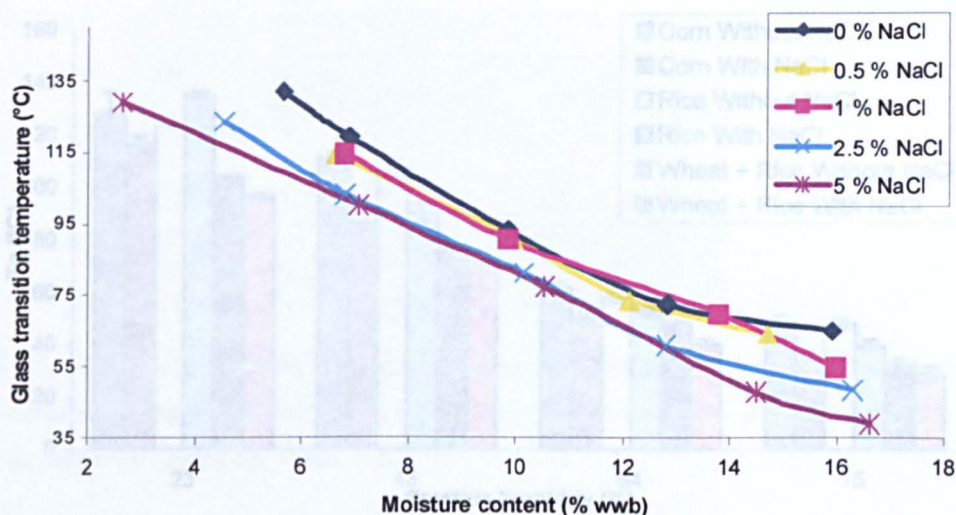


Figure 6.3: Glass transition temperature (as measured by PTA using the maximum value of the derivative curve) of model systems containing pregelatinised starch and NaCl (0 to 5 % dwb) versus the moisture content of the system.

When NaCl was added to pregelatinised starch, it significantly increased the

The glass transition temperature of systems composed of pregelatinised starch and sodium chloride between 0 and 1 % (dwb) was significantly different from Tg of the same systems containing 2.5 or 5 % NaCl (dwb). Indeed, when NaCl concentration increased from 1 % to 2.5 %, the glass transition temperature decreased on average by 10 °C regardless of the moisture content of the system. Similar trends were observed with Tg measurements of the model systems by DSC (data not shown).

the moisture retention of the system, colour formation should then be correlated to the

The glass transition temperature of breakfast cereal products stored for 3 weeks at several relative humidities was also influenced by the presence of NaCl. Tg was significantly lower ($p < 0.05$) when NaCl was present in the product compared to the same product without NaCl for relative humidities of 43 %, 64 % and 75 % (Figure 6.4). Similar results were obtained by DSC (data not shown). It should be noted that in Figure 6.4, Tg was plotted versus the relative humidity at which the product was stored and not versus the moisture content of the product. As NaCl is hygroscopic, the moisture content of a product containing NaCl tends to be higher, as previously observed. Water is also a known plasticiser. Hence, the higher moisture content of products containing NaCl can also be responsible, together with NaCl, for the lowering of Tg (Figure 6.4).

these systems is presented in Figure 6.3.

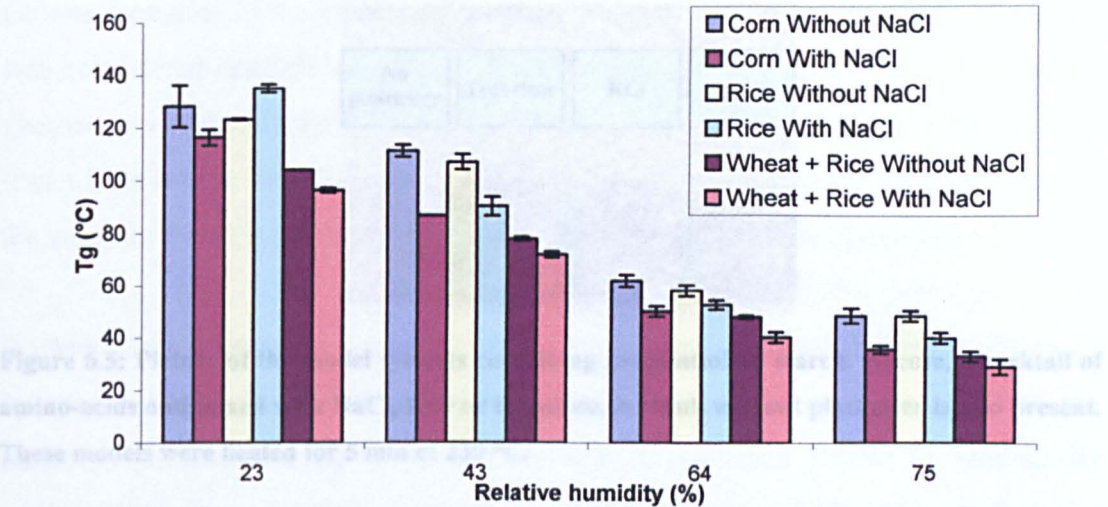


Figure 6.4: Glass transition temperature (as measured by PTA) of breakfast cereal products, with and without NaCl, stored for 3 weeks at several relative humidities.

When NaCl was added to pregelatinised starch, it significantly increased the moisture sorption after storage and decreased the glass transition temperature of the system. The influence of NaCl on colour formation in model systems composed of pregelatinised starch, amino-acids and glucose might be due to these system changes, as explained previously.

If the influence of NaCl on colour was indeed due to changes in the Tg or the moisture retention of the system, colour formation should then be correlated to the hygroscopic behaviour and the plasticising ability of the same models mixed with other plasticisers than NaCl. These hypotheses were tested and the results are presented in the following section.

6.4.3. Replacement of NaCl by other plasticisers

Model systems with pregelatinised starch, glucose and the cocktail of amino-acids were mixed with several plasticisers: NaCl, KCl or trehalose (Table 6.3). One model system was made without plasticiser. These systems were heated up to 5 min at 230 °C. Colour measurements were then performed on each model. A picture of these systems is presented in Figure 6.5.

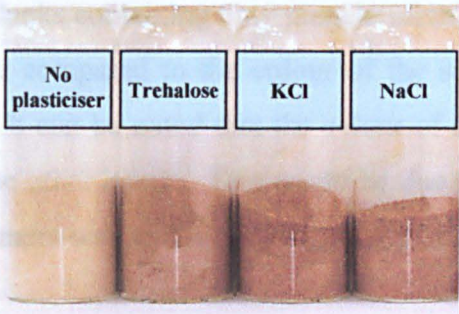


Figure 6.5: Picture of the model systems containing pregelatinised starch, glucose, a cocktail of amino-acids and mixed with NaCl, KCl or trehalose. A blank without plasticiser is also present. These models were heated for 5 min at 230 °C.

Presence of plasticiser in the model systems (KCl, NaCl or trehalose) always led to darker products after 5 min heating at 230 °C ($p<0.05$). However, no significant differences could be observed within the plasticisers. The use of trehalose, KCl or NaCl led to the same L^* values ($p>0.05$).

To check whether the plasticiser in itself generated colour, blank systems with only pregelatinised starch and the plasticiser were made. They were heated under the same conditions as the model systems and colour formation was monitored. Results are presented in Figure 6.6.

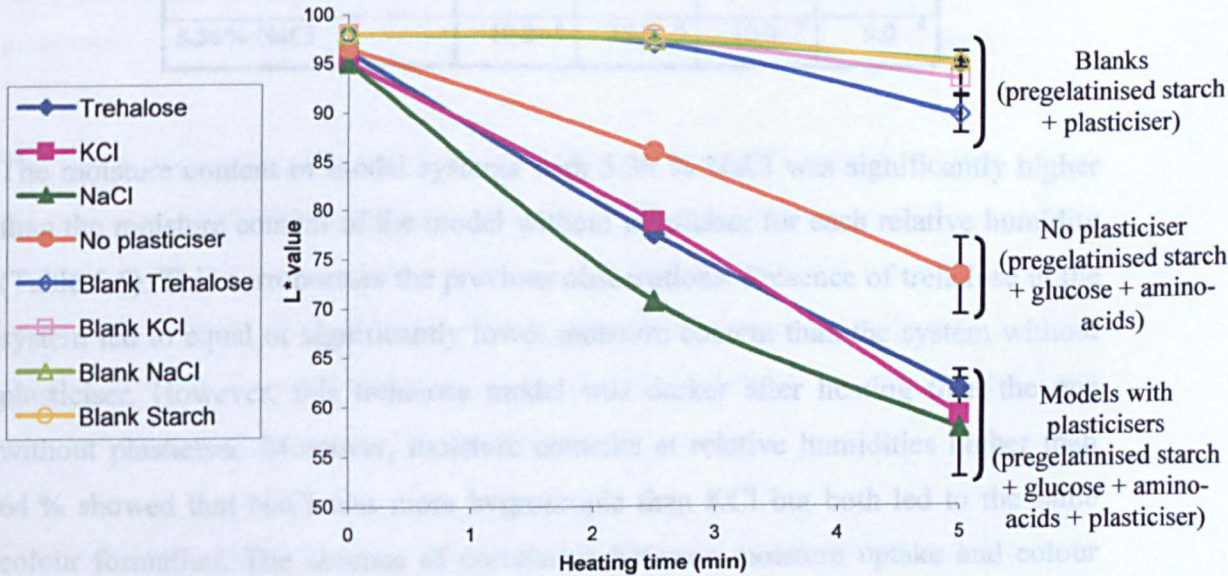


Figure 6.6: L^* value of the model systems and the blanks containing different plasticisers versus heating time at 230 °C. Triplicates were made for a heating time of 5 min.

Colour formation in the blanks containing only pregelatinised starch and a plasticiser was considered negligible compared to the colour of the same system with added glucose and amino-acids. It can be noted that the colour of the blank with trehalose was a little darker than the other blanks. This might be due to the caramelisation of the sugar as this heat treatment was severe (heating temperature above 200 °C).

If there was a correlation between colour formation of a system and the plasticiser's hygroscopic behaviour or its ability to decrease the glass transition temperature, the moisture sorption and Tg of all models with a plasticiser should be statistically equivalent as the colour formation was the same. However, it should be statistically different from the model without plasticiser, as this was lighter in colour ($p < 0.05$). Moisture content of the model systems with and without plasticiser was measured after 3 weeks equilibration at several relative humidities (Table 6.5).

Table 6.5: Moisture content (% wwb) of model systems after equilibration at several relative humidities. Measurements done in triplicate. The letters by the moisture values indicate significant differences between the samples within a specific relative humidity ($p < 0.05$).

Relative humidities	75%	64%	43%	23%
No plasticiser	15.8 ^a	13.9 ^b	10.4 ^b	8.1 ^c
25% Trehalose	16.2 ^a	12.9 ^a	9.3 ^a	7.3 ^b
5.36% KCl	16.9 ^b	14.3 ^{b,c}	11.2 ^c	8.9 ^d
5.36% NaCl	19.2 ^c	15.2 ^d	10.9 ^c	9.0 ^d

The moisture content of model systems with 5.36 % NaCl was significantly higher than the moisture content of the model without plasticiser for each relative humidity (Table 6.5). This corroborates the previous observations. Presence of trehalose in the system led to equal or significantly lower moisture content than the system without plasticiser. However, this trehalose model was darker after heating than the one without plasticiser. Moreover, moisture contents at relative humidities higher than 64 % showed that NaCl was more hygroscopic than KCl but both led to the same colour formation. The absence of correlation between moisture uptake and colour formation among these 4 samples demonstrated that the hygroscopic behaviour of a plasticiser cannot be correlated to the colour formation of the corresponding system after heating at 230 °C for 5 min. This might be due to the fact that the ability of a

component to sorb water during long term storage is not necessarily linked to the ability to retain water from evaporation during heating.

To check whether the presence of NaCl in breakfast cereals influenced water evaporation, weight loss from wheat doughs (with an initial moisture content around 20 % wwb) heated in a TGA (from 25 to 190 °C at 10 °C/min) was followed. Results are presented in Figure 6.7.

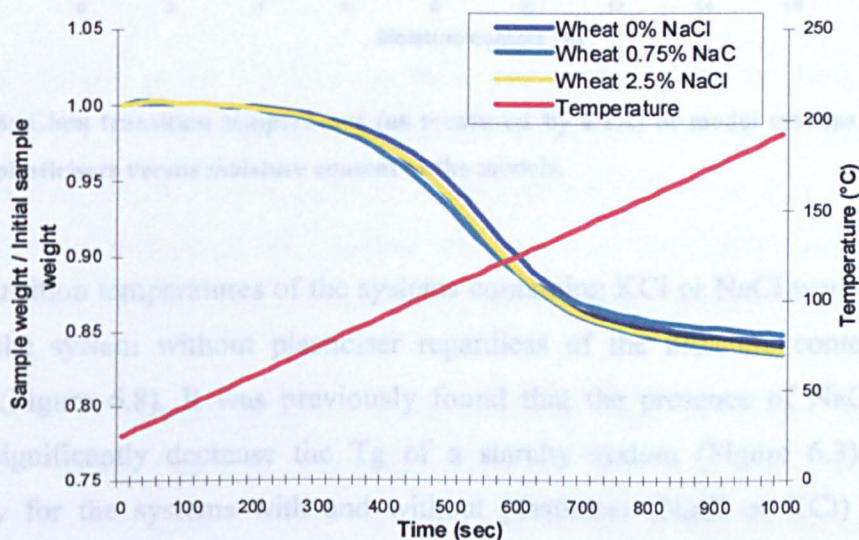


Figure 6.7: Sample weight / sample initial weight ratio of wheat doughs containing different NaCl concentrations and heated with a TGA (temperature raised according to the first programme presented in section 3.2.2.7). Samples measured and presented in duplicates.

The wheat doughs were heated from 25 °C to 190 °C. It was considered that, during this heat treatment, only water was responsible for weight loss (no significant volatile formation and release). The level of NaCl in these products did not seem to affect water evaporation (Figure 6.7). Hence, this measurement confirmed that the influence of NaCl on colour formation was not dominated by any altered water retention of the system during the heat treatment.

Glass transition temperatures of the model systems with and without plasticiser were measured after 3 weeks equilibration at several relative humidities and are presented in Figure 6.8.

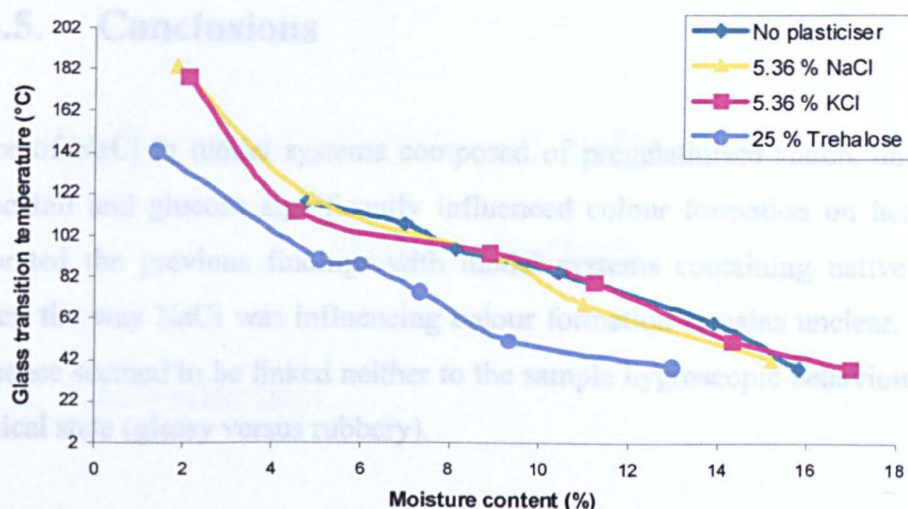


Figure 6.8: Glass transition temperature (as measured by PTA) of model systems containing different plasticisers versus moisture content of the models.

Glass transition temperatures of the systems containing KCl or NaCl were similar to that of the system without plasticiser regardless of the moisture content of the product (Figure 6.8). It was previously found that the presence of NaCl or KCl should significantly decrease the Tg of a starchy system (Figure 6.3). The Tg similarity for the systems with and without plasticiser (NaCl or KCl) for these models can be explained by the presence of glucose and amino-acids, which are low molecular weight molecules. All these molecules might tend to decrease Tg, like NaCl and KCl would do, hence they might mask the impact of these salts on Tg. Tg of the models with trehalose was lower than all the other models at all the moisture contents examined. This is likely to be due to the high trehalose concentration (25 % dwb), compared to the NaCl and KCl concentrations used (5.36 % dwb). It was found that both models containing NaCl and KCl led to darker products than the model without plasticiser, even though the Tg of these three types of models was similar. Moreover, the glass transition temperature of the model with trehalose was around 30 °C lower than the other systems (with NaCl, KCl or without plasticiser) at comparative moisture contents. However, the colour generated by the trehalose system was significantly darker compared to the model without plasticiser, but gave equivalent colour to the systems with NaCl or KCl. This experiment demonstrated that colour generation of this type of system heated at 230 °C did not seem to be primarily influenced by the change in the glass transition temperature.

6.5. Conclusions

Presence of NaCl in model systems composed of pregelatinised starch, an amino-acid cocktail and glucose significantly influenced colour formation on heating. It corroborated the previous findings with model systems containing native starch. However, the way NaCl was influencing colour formation remains unclear. Indeed, its influence seemed to be linked neither to the sample hygroscopic behaviour nor to its physical state (glassy versus rubbery).

Chemical changes in the Maillard reactions pathways might occur because of the presence of NaCl or other molecules such as KCl or trehalose, which could explain their impact on colour. Indeed, Gokmen and Senyuva (2007) previously reported that the presence of cations in the reaction mixture limits the Schiff base formation, which are primary compounds formed during the Maillard reactions (Figure 2.12).

The influence of caramelisation on colour formation might have been neglected in this study. Indeed, this type of reaction does not involve at first a bimolecular condensation. Hence, the mobility and the diffusion of the reactant are less crucial for colour formation via caramelisation. It was previously demonstrated that the overall kinetics of caramelisation was not related to the physical state of the matrix (Claude and Ubbink, 2006). This could explain why the physical state of the product did not seem to be the key factor for colour formation if most of the colour was due to caramelisation.

To understand the role of NaCl on colour formation, it is necessary to simplify the model systems. As colour formation can be due to both caramelisation and Maillard reactions, the models were broken down into different parts to be able to comprehend the influence of NaCl on each compound. Mixtures of NaCl with glucose or with glucose and amino-acids allowed the study of NaCl on caramelisation and Maillard reactions separately (Chapter 8). The next chapter used model systems composed only of starch and NaCl to observe the impact of NaCl on this particular material during a heat treatment (Chapter 7).

7. Influence of several salts on starch degradation

7.1. Introduction

In the previous chapters, a model system mimicking breakfast cereals regarding colour, some residual volatiles and acrylamide formation was developed. This model was used to observe the influence of NaCl on these parameters. It was found that presence of NaCl enhanced colour formation and decreased acrylamide content. These observations were confirmed by some experiments made with breakfast cereal products. Two hypotheses were made regarding the way NaCl might influence Maillard and caramelisation reactions, hence colour and acrylamide formation. They concerned the influence of NaCl on molecule mobility and diffusion. However, experiments using model systems showed that these hypotheses were not valid.

As the influence of NaCl on Maillard and caramelisation reactions remained unclear, a simplification of the model system was needed. Indeed, in the model system used, both types of reactions could happen. However, the influence of NaCl might be different on Maillard reactions compared to caramelisation reactions. As colour formation can be the result of both, the study of each of these reactions separately was needed. The model system was therefore broken down into different parts, and each part was the subject of a different study. A model containing only starch and NaCl focused on the influence of NaCl on possible starch degradations. A model containing only glucose and NaCl indicated the influence of NaCl on caramelisation reactions, while a model with glucose, amino-acids and NaCl demonstrated some possible impacts of NaCl on Maillard reactions.

In this chapter, model systems containing only starch and NaCl were used to limit the observations of the influence of NaCl on starch and some possible degradation during toasting. If NaCl does have an impact on colour during starch toasting, a

possible impact of NaCl on starch and its degradation into glucose molecules (which could then caramelize) should be considered. The studies focusing on the impact of NaCl on colour of systems composed of glucose or glucose and amino-acids are presented in Chapter 8.

7.2. Aims

The first aim of this study was to observe whether NaCl had an influence on starch during toasting. Model systems containing only starch, from several botanical origins, native or gelatinised, were mixed with NaCl and were toasted in a similar way as the model systems used previously. Colour formation was then monitored. In the case of models containing native starch, the starch granule crystallinity and swelling power after heating was checked using several methods. When the models contained pregelatinised starch, the heat treated models were dissolved and the viscosity of the solution was measured to evaluate some possible molecular weight changes of the starch polymers. X-ray measurements of starchy systems containing NaCl gave some indications concerning the interaction between this salt and the starch. The second objective of this study was to observe whether other types of salts had the same impact as NaCl on starch crystallinity loss during a toasting treatment and to try to understand the mechanism.

7.3. Influence of NaCl on native starch during toasting

7.3.1. System preparation

- Influence of NaCl on native waxy maize, cassava and potato starch during toasting

Model systems containing native starch (waxy maize, potato or cassava starches) and NaCl (0.00, 0.75, 1.00, 2.00 and 4.00 % of NaCl dwb), with a moisture content of

20 % (wwb) were prepared according to the procedure presented in section 3.2.1.1. Aliquots of 60 g were placed in open aluminium containers and were heated in an oven for 45 min at 230 °C.

- Influence of NaCl on native waxy maize starch during toasting: experimental design

The model system containing native waxy maize starch and 2.00 % NaCl (dwb), with a moisture content of 20 % (wwb) was prepared in a larger quantity to be used in an experimental design. Aliquots of 50 g were heated for different times (between 15 and 90 min) and temperatures (between 180 °C and 230 °C) according to the design presented in Table 7.1

Table 7.1: Experimental design used to study the influence of 2.00 % NaCl (dwb) on starch granule degradation during toasting using different heating times and temperatures.

Run Number	Heating time (min)	Heating temperature (°C)
1	52.50	205.00
2	90.00	180.00
3	40.00	230.00
4	71.25	192.50
5	15.00	230.00
6	33.75	192.50
7	52.50	205.00
8	90.00	230.00
9	90.00	213.33
10	65.00	230.00
11	52.50	180.00
12	90.00	180.00
13	15.00	196.67
14	90.00	196.67
15	15.00	180.00
16	15.00	213.33
17	90.00	230.00
18	15.00	180.00
19	52.50	205.00

- Intrinsic viscosity measurements

To measure the intrinsic viscosity of the native waxy maize starch toasted with NaCl, the samples were mixed with aqueous solutions of KOH (5 M). Samples containing 0.00, 0.75 and 1.00 % NaCl (dwb) were mixed with the KOH solution at a concentration of 200 mg of starch / ml of mixture, the samples with 2.00 and 4.00 % NaCl at a concentration of 400 mg of starch / ml of mixture, and the non toasted native waxy maize starch at a concentration of 50 mg of starch / ml of mixture. After stirring the mixtures for 2.5 hours until complete dissolution of the starchy sample, the resulting solutions were then serially diluted in distilled water to obtain the final starch concentrations presented in Table 7.2.

Table 7.2: Diluted starch solution concentrations used to determine the starch intrinsic viscosity.

Sample	Final starch concentrations (g/ml of final solution)
Non toasted native waxy maize starch	0.002, 0.004, 0.008, 0.012 and 0.016
Waxy maize starch toasted with 0.00 % NaCl (dwb)	0.012, 0.016, 0.020, 0.024 and 0.028
Waxy maize starch toasted with 0.75 % NaCl (dwb)	0.012, 0.016, 0.020, 0.024 and 0.028
Waxy maize starch toasted with 1.00 % NaCl (dwb)	0.024, 0.028, 0.032, 0.036 and 0.040
Waxy maize starch toasted with 2.00 % NaCl (dwb)	0.040, 0.044, 0.048, 0.052 and 0.056
Waxy maize starch toasted with 4.00 % NaCl (dwb)	0.064, 0.068, 0.072, 0.076 and 0.080

Intrinsic viscosity measurements were performed as presented in section 3.2.2.11. The amount of NaCl present in the solutions was different for each sample. However, NaCl was considered to have a negligible influence on the starch solution viscosity, as previously shown by other authors (Valles-Pamies et al., 1997). Moreover, the KOH concentration varied between 0.6 and 1.0 M in the diluted solutions due to the different dilutions applied to each sample. The viscosity of a solution with a KOH concentration of 0.8 M was taken into account for relative viscosities calculations. It was measured that such solution had a viscosity of 1.10 mPa.s at 20 °C and under similar measurement conditions as those applied for the samples.

The viscosity values reported are the averages of 4 measurements done per angle performed for 3 measuring angles (50, 60 and 70 °, see section 3.2.2.11). Similar

viscosity values were obtained at each measuring angle. This showed that these waxy maize starch samples were not significantly shear thinning under the conditions applied, as previously reported by Durrani and Donald (2000).

7.3.2. Results and discussion

7.3.2.1. Models with waxy maize, cassava and potato starch

Model systems containing native starch (waxy maize, cassava or potato) and NaCl between 0 and 4 % (dwb) with an initial moisture content of 20 % (wwb) were heated in an oven for 45 min at 230 °C. A picture of these samples is represented in Figure 7.1. Colour measurements were then performed on each sample (Figure 7.2) according to the method presented in section 3.2.2.2. Regardless of the type of native starch used, a linear correlation was observed between the lightness of the system after the heat treatment (L^* value) and the NaCl concentration. The darkness of the sample increased proportionally with the NaCl concentration regardless of the starch botanical origin. This observation follows the same trend as for the model systems (with glucose and amino-acids) and the breakfast cereal products studied in Chapter 5 and 6.



Figure 7.1: Picture of the model systems containing native starch (waxy maize, cassava or potato) and NaCl (0 to 4 % dwb) heated at 230 °C for 45 min in an open container.

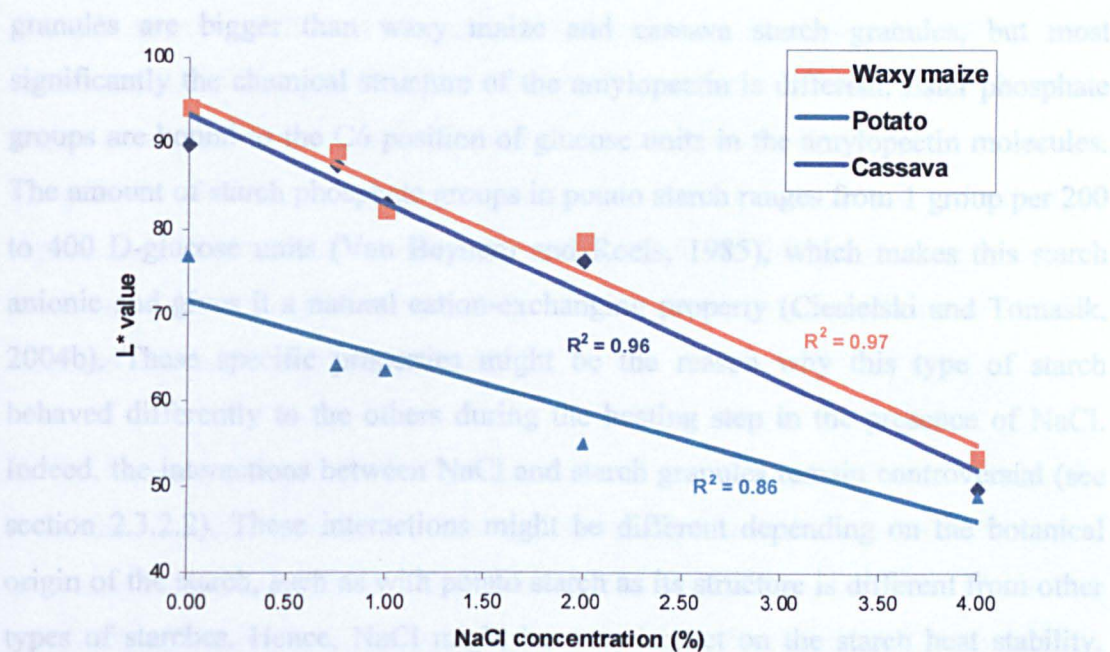


Figure 7.2: Influence of NaCl concentration (% dwb) on the L* value of model systems containing native starch (waxy maize, cassava or potato) and NaCl heated at 230 °C for 45 min in an open container.

Native cassava and waxy maize starch were both developing similar colour for a given NaCl concentration when heated at 230 °C. According to the literature, cassava starch contains twice to 3 times less residual proteins compared to waxy maize starch (0.1 % versus 0.25 % respectively) (Van Beynum and Roels, 1985). As similar colour intensities were developed, colour formation could be mainly due to caramelisation rather than Maillard reactions, which necessitate the presence of proteins or amino-acids, hence should be influenced by the protein level.

When there was no NaCl in the system, the potato starch samples were darker after the heat treatment compared to the 2 other types of starch (Figure 7.2), even though its residual protein level was considered as low as cassava starch (Van Beynum and Roels, 1985). When NaCl was added to the potato starch, its impact on colour formation was lower than for the other types of starch as the slope of the linear trend curve was smaller. Overall, the colour of potato starch samples heated with NaCl concentrations between 0 and 4 % (dwb) was darker than for samples with cassava or waxy maize starch. This different behaviour of potato starch might be explained by its unique properties compared to other types of starches. Indeed, the potato starch

granules are bigger than waxy maize and cassava starch granules, but most significantly the chemical structure of the amylopectin is different. Ester phosphate groups are bound to the C6 position of glucose units in the amylopectin molecules. The amount of starch phosphate groups in potato starch ranges from 1 group per 200 to 400 D-glucose units (Van Beynum and Roels, 1985), which makes this starch anionic and gives it a natural cation-exchanging property (Ciesielski and Tomasik, 2004b). These specific properties might be the reason why this type of starch behaved differently to the others during the heating step in the presence of NaCl. Indeed, the interactions between NaCl and starch granules remain controversial (see section 2.3.2.2). These interactions might be different depending on the botanical origin of the starch, such as with potato starch as its structure is different from other types of starches. Hence, NaCl might have an impact on the starch heat stability, which could be different depending on its botanical origin.

The pH of the suspensions composed of 1.00 g of heated model system and 9.00 g of distilled water decreased as the NaCl concentration increased (Figure 7.3).

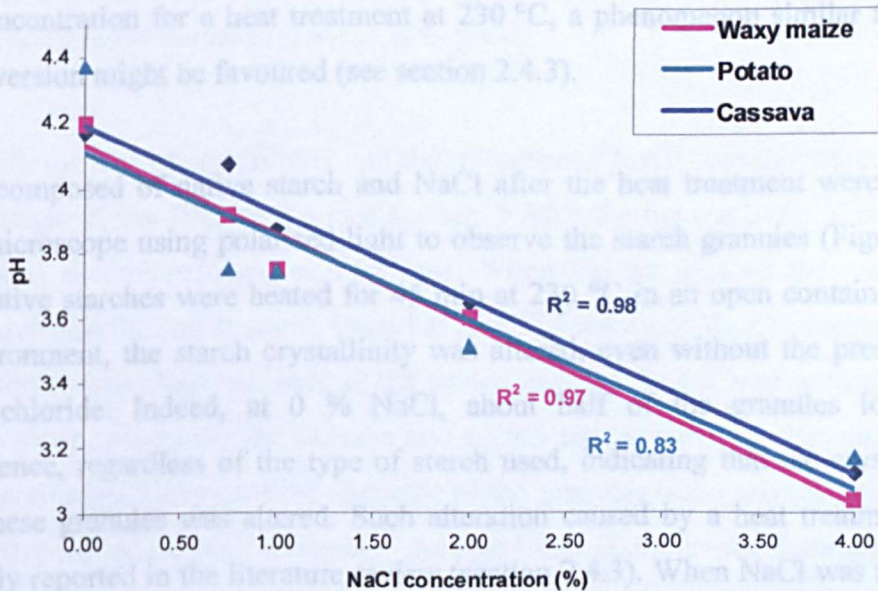


Figure 7.3: Influence of NaCl concentration (% dwb) during a heat treatment with starch (230 °C for 45 min) on the pH of a suspension of 1.00 g of model systems (containing native starch (waxy maize, cassava or potato) and NaCl heated at 230 °C for 45 min in an open container) mixed with 9.00 g of distilled water.

The pH decrease followed the same trend as colour formation versus the NaCl concentration. It was previously mentioned that colour formation might be mainly due to caramelisation reactions. These reactions lead to the formation of H^+ . Hence the pH of a system undergoing caramelisation reactions decreases, which might explain the pH change observed in Figure 7.3. However, this pH decrease was not a proof that colour formation was mainly due to caramelisation reactions as Maillard reactions also lead to the formation of H^+ .

At the temperature used (230 °C), hydrolysis of starch might occur, leading to the formation of smaller molecules such as glucose, as presented in the literature review in section 2.4.3 (Aggarwal and Dollimore, 1996; Tomasik et al., 1989; Davidson et al., 1984). In the systems shown in Figure 7.2, colour formation should most likely be due to caramelisation rather than Maillard reactions (quasi absence of proteins). Colour formation of these systems, containing only NaCl and native starch, could indicate a possible impact of this salt on the starch macromolecular assembly and an eventual formation of glucose during the heat treatment leading to more caramelisation. Indeed, as the pH of the system decreased to a value around 3 at high NaCl concentration for a heat treatment at 230 °C, a phenomenon similar to starch pyroconversion might be favoured (see section 2.4.3).

Models composed of native starch and NaCl after the heat treatment were viewed with a microscope using polarised light to observe the starch granules (Figure 7.4). When native starches were heated for 45 min at 230 °C in an open container and a dry environment, the starch crystallinity was altered, even without the presence of sodium chloride. Indeed, at 0 % NaCl, about half of the granules lost their birefringence, regardless of the type of starch used, indicating that the crystallinity within these granules was altered. Such alteration caused by a heat treatment was previously reported in the literature review (section 2.4.3). When NaCl was added at a level of 2 % to the native starches, almost all the granules lost their Maltese cross during the heat treatment. No birefringence could be observed in samples that contained 4 % NaCl and were heated, regardless of the starch botanical origin. Micrographs shown in Figure 7.4 demonstrate that a heat treatment of 45 min at 230 °C altered the native starch granules crystallinity; the addition of NaCl enhanced this loss of crystallinity.

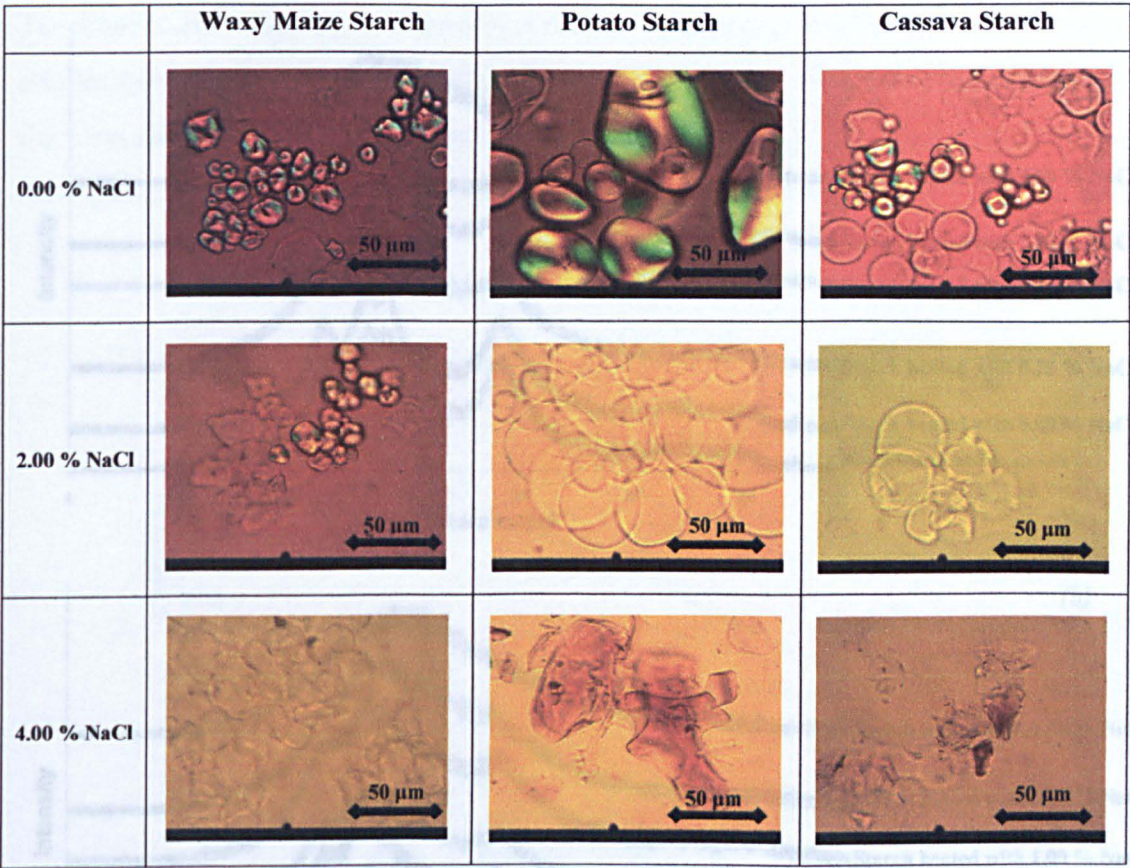


Figure 7.4: Microscopic observations using polarised light of native starch (waxy maize, cassava and potato) mixed with NaCl (0 to 4 % dwb) and heated at 230 °C for 45 min in an open container; initial model moisture content of 20 % (wwb).

To quantify the loss of crystallinity and the influence of NaCl, samples were analysed by wide angle X-ray (Figure 7.5, see section 3.2.2.9 for the method). X-ray results corroborated the microscopic observations. When the patterns of the non heated native starch and the native starch heated at 230 °C for 45 min were compared, a loss of crystallinity was observed. Indeed, the peaks obtained for the heat treated samples were less sharp, especially for cassava and potato starch, meaning that under these heating conditions, the crystalline structure of the granules was reduced. When NaCl was added prior to the heat treatment, the sharpness of the peaks decreased as the NaCl concentration increased, regardless of the type of starch used, showing a decrease of the granule crystallinity. At 4 % NaCl, the crystallinity of cassava, potato and waxy maize starch granules was lost (Figure 7.5). The diffractograms indicated an amorphous material. A sharp peak at a measuring angle of 32 ° was observed at 4 % NaCl for the cassava and potato starch samples, this is indicative of NaCl crystals.

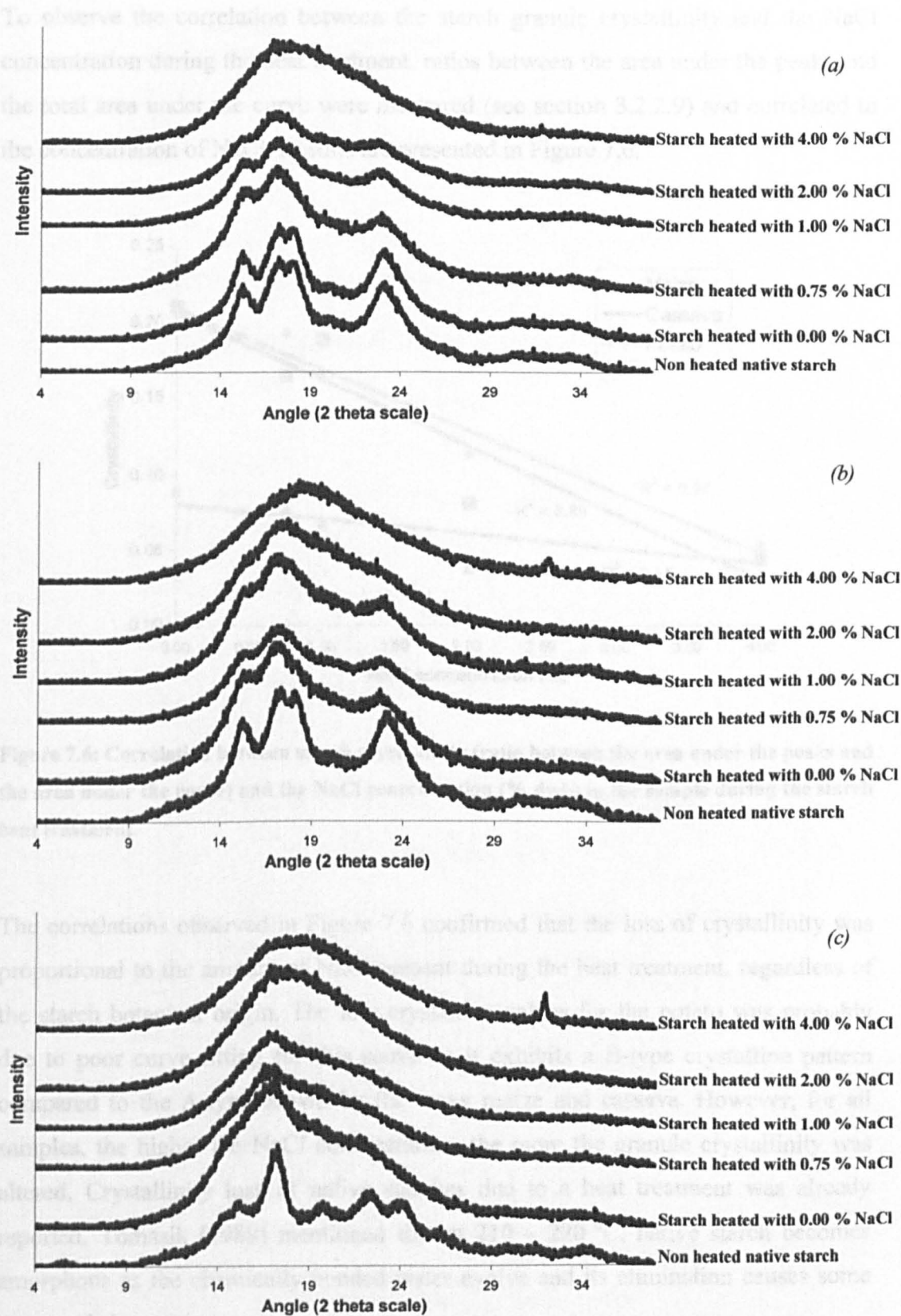


Figure 7.5: Wide angle X-ray patterns of native starch (waxy maize (a), cassava (b) and potato (c)) mixed with NaCl (0 to 4 % dwb), not heated or heated at 230 °C for 45 min; measurements performed at angles between 4 and 38 ° (2θ) with a step size of 0.02 ° and a time per step of 3 s (curves have been displaced on the y axis for clarity purposes).

To observe the correlation between the starch granule crystallinity and the NaCl concentration during the heat treatment, ratios between the area under the peaks and the total area under the curve were measured (see section 3.2.2.9) and correlated to the concentration of NaCl. Results are presented in Figure 7.6.

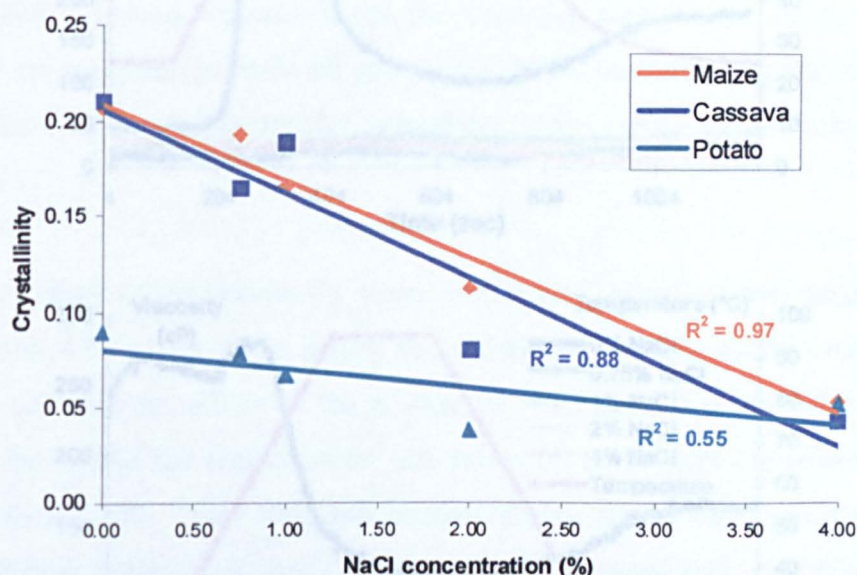


Figure 7.6: Correlation between starch crystallinity (ratio between the area under the peaks and the area under the curve) and the NaCl concentration (% dwb) in the sample during the starch heat treatment.

The correlations observed in Figure 7.6 confirmed that the loss of crystallinity was proportional to the amount of NaCl present during the heat treatment, regardless of the starch botanical origin. The low crystalline values for the potato was probably due to poor curve fitting for this starch as it exhibits a B-type crystalline pattern compared to the A-type shown by the waxy maize and cassava. However, for all samples, the higher the NaCl concentration, the more the granule crystallinity was altered. Crystallinity loss of native starches due to a heat treatment was already reported. Tomasik (1989) mentioned that at 210 – 220 °C, native starch becomes amorphous as the chemically bonded water evolve and its elimination causes some structural changes.

The swelling capacities of the heat treated starches and their aptitude to increase a solution viscosity were measured by RVA (Figure 7.7, method in section 3.2.2.8).

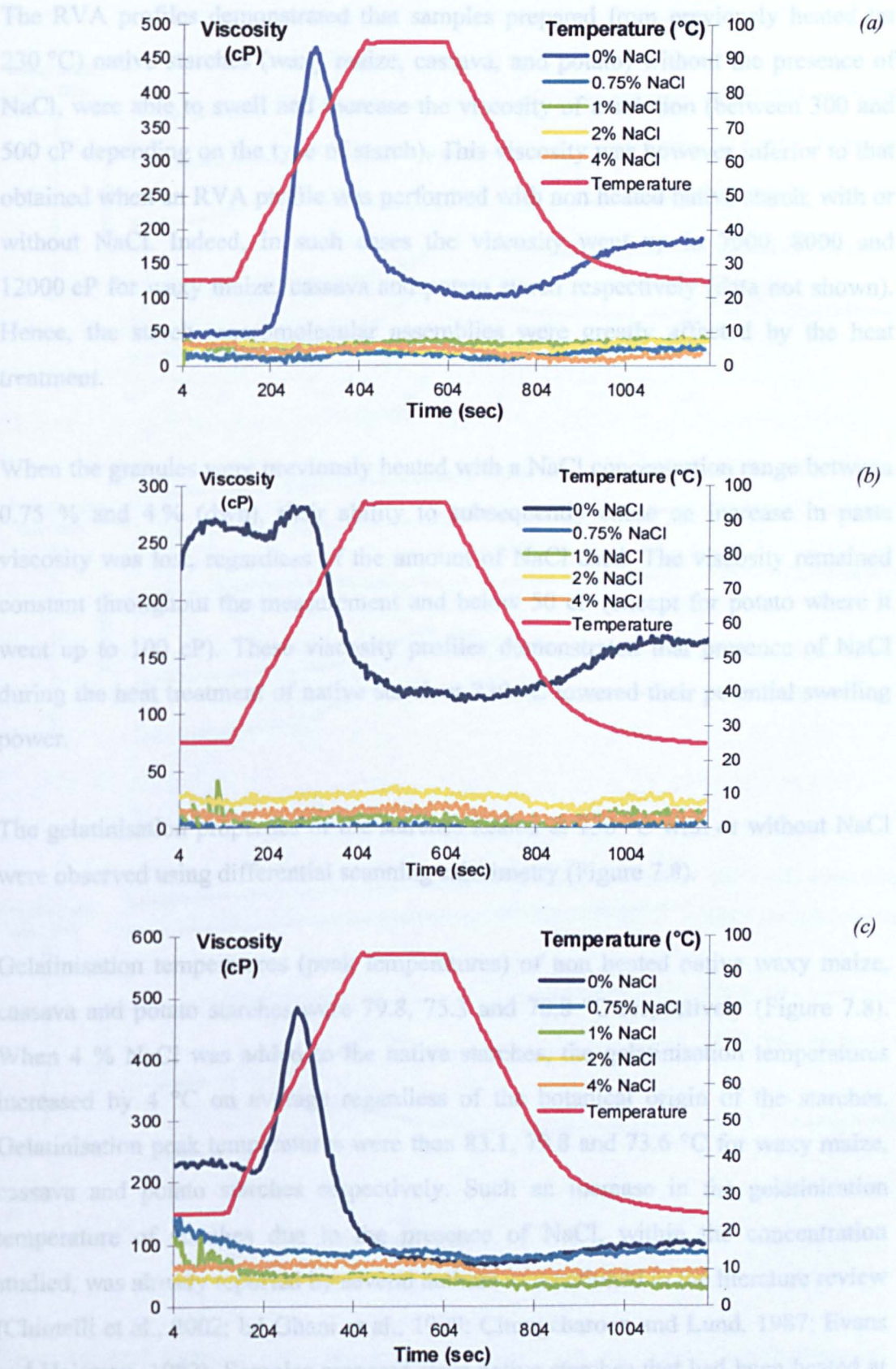


Figure 7.7: RVA profiles of 4.00 g of model system (dwb) mixed with distilled water (total weight of 28.00 g). Models consisted of native starch (waxy maize (a), cassava (b) and potato (c)) mixed with NaCl (0 to 4 % dwb) that were heated at 230 °C for 45 min.

The RVA profiles demonstrated that samples prepared from previously heated (at 230 °C) native starches (waxy maize, cassava, and potato) without the presence of NaCl, were able to swell and increase the viscosity of a solution (between 300 and 500 cP depending on the type of starch). This viscosity was however inferior to that obtained when an RVA profile was performed with non heated native starch, with or without NaCl. Indeed, in such cases the viscosity went up to 7000, 8000 and 12000 cP for waxy maize, cassava and potato starch respectively (data not shown). Hence, the starch macromolecular assemblies were greatly affected by the heat treatment.

When the granules were previously heated with a NaCl concentration range between 0.75 % and 4 % (dwb), their ability to subsequently cause an increase in paste viscosity was lost, regardless of the amount of NaCl used. The viscosity remained constant throughout the measurement and below 50 cP (except for potato where it went up to 100 cP). These viscosity profiles demonstrated that presence of NaCl during the heat treatment of native starch at 230 °C lowered their potential swelling power.

The gelatinisation properties of the starches heated at 230 °C with or without NaCl were observed using differential scanning calorimetry (Figure 7.8).

Gelatinisation temperatures (peak temperatures) of non heated native waxy maize, cassava and potato starches were 79.8, 75.3 and 70.8 °C respectively (Figure 7.8). When 4 % NaCl was added to the native starches, the gelatinisation temperatures increased by 4 °C on average regardless of the botanical origin of the starches. Gelatinisation peak temperatures were then 83.1, 79.8 and 73.6 °C for waxy maize, cassava and potato starches respectively. Such an increase in the gelatinisation temperature of starches due to the presence of NaCl, within the concentration studied, was already reported by several authors as mentioned in the literature review (Chiotelli et al., 2002; bd Ghani et al., 1999; Chungcharoen and Lund, 1987; Evans and Haisman, 1982). Samples prepared from native starches that had been heated at 230 °C for 45 min without NaCl showed no gelatinisation peaks between 70 and 85 °C. This confirmed previous observations made with the microscope, the X-ray and the RVA, being that the heat treatment affects the starch granules.

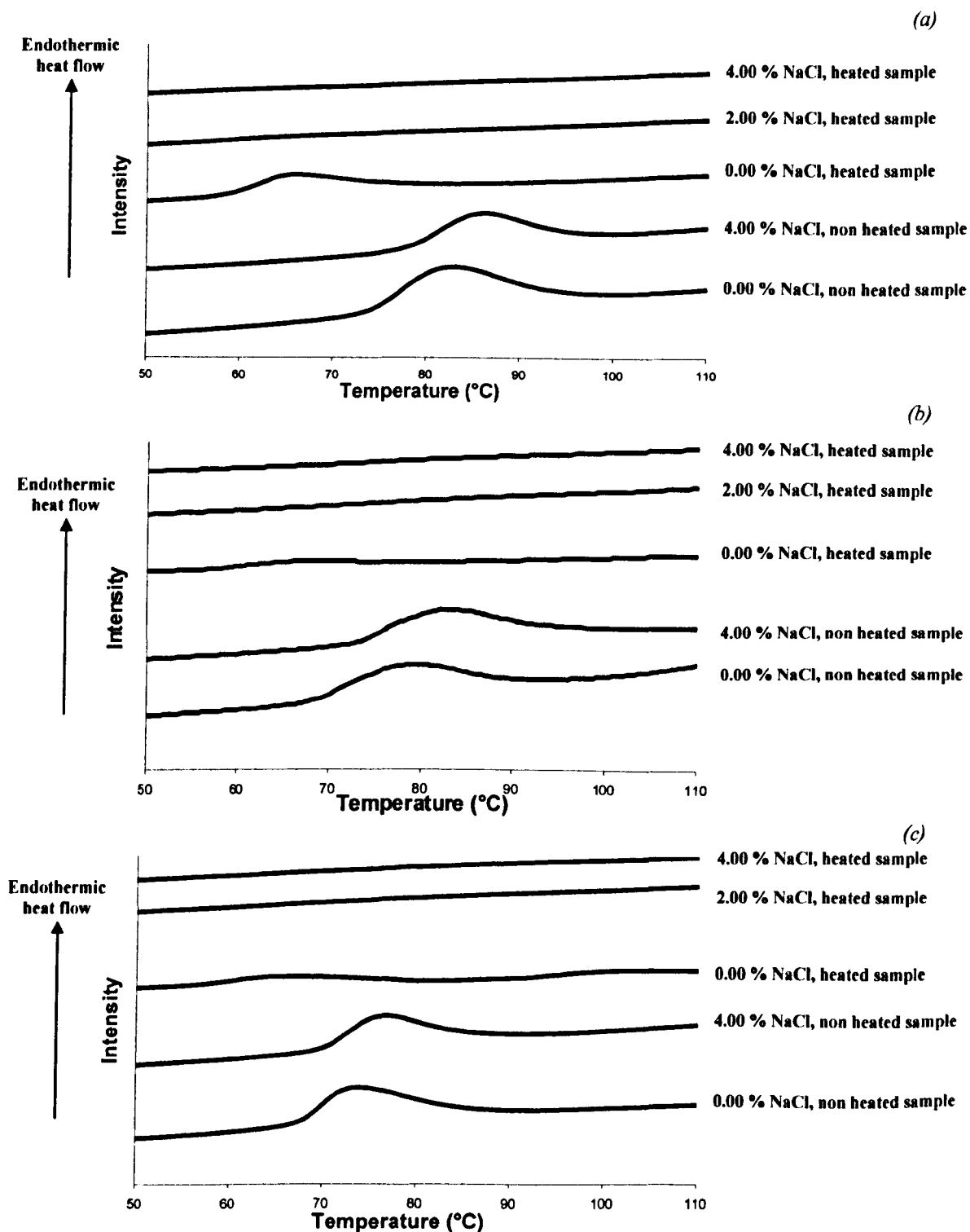


Figure 7.8: DSC profiles of native starch samples (waxy maize (a), cassava (b) and potato (c)) mixed with NaCl (0 to 4 % dwb) not heated or heated at 230 °C for 45 min. DSC profiles performed with a starch/water ratio of 1/3 (curves have been displaced on the y axis for clarity).

However, an endothermic peak was observed for temperatures between 63 and 66 °C for the samples made from native cassava, potato and waxy maize starch and heated without NaCl. This peak might be due to the melting of some retrograded starch that might have formed after the heat treatment at 230 °C (Thomas, 1999).

No gelatinisation peaks were observed for the samples formed from native starch samples previously heated with 2 or 4 % NaCl, which demonstrated that the starch granules were altered by the heat treatment. Moreover, no peaks were observed between 63 and 66 °C. The absence of peaks within this temperature range may demonstrate that the starch granule was more degraded with the presence of NaCl during the heat treatment at 230 °C, and thus could not form crystallites observable by DSC.

The intrinsic viscosity of the waxy maize starch samples heated with and without NaCl, dissolved in KOH 5 M and then diluted in distilled water, was measured. Results are presented in Figure 7.9. The extrapolations for a starch concentration of 0 g/ml of solution were made for each sample. Averages of the Kraemer and the Huggins extrapolation (see section 3.2.2.11) were then calculated and correspond to the intrinsic viscosity of the sample.

The intrinsic viscosity of the non heated native waxy maize starch solution was found to be around 77 ml/g. Such a value is significantly lower than values reported in the literature, which are between 107 and 115 ml/g (Singh et al., 2006) for waxy maize starch dissolved in DMSO. This difference might be due to the severe alkaline treatment of the samples to ensure their dissolution, and/or to the different solvent used. The intrinsic viscosity of the non heated starch sample was significantly higher than for the starch sample heated without NaCl ($[\eta] = 53$ ml/g). This observation confirmed that the heat treatment in itself degraded the starch macromolecules.

The intrinsic viscosity results obtained for the starch samples heated at 230 °C for 45 min with NaCl (concentration between 0 and 4 % dwb) were plotted as a function of the NaCl concentration (Figure 7.10). It was observed that the intrinsic viscosity decreased non-linearly as the NaCl concentration increased. This might show that the presence of NaCl during the heat treatment could have accelerated the starch

polymers' degradation, leading to smaller molecules, and these would have a smaller hydrodynamic volume.

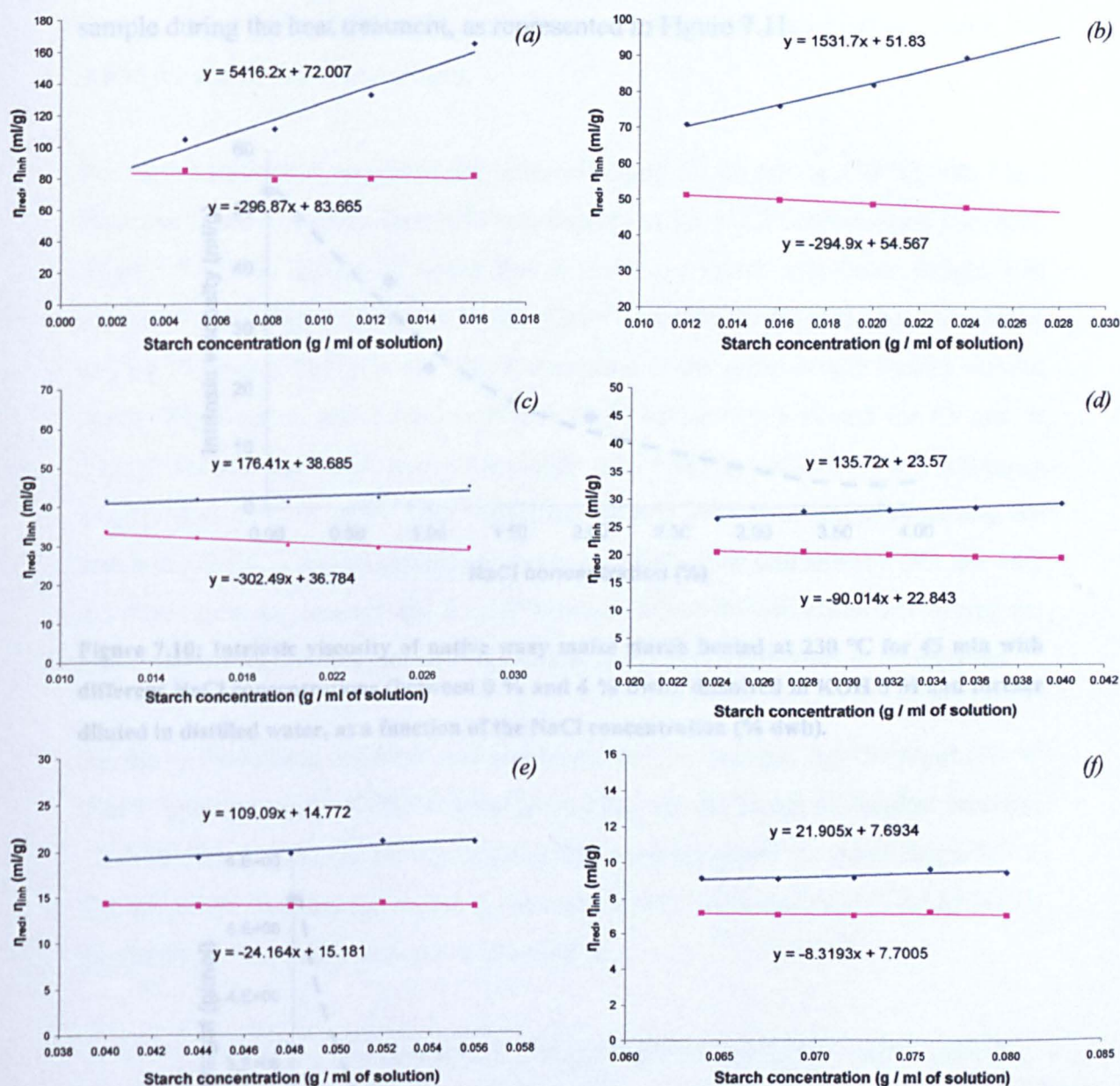


Figure 7.9: Reduced (\blacklozenge) and inherent (\blacksquare) viscosity of non heated waxy maize starch samples (a) or waxy maize starch heated at 230 °C for 45 min with different NaCl concentrations (dwb): 0 % (b), 0.75 % (c), 1 % (d), 2 % (e) and 4 % (f). These samples were dissolved in KOH 5 M and further diluted in distilled water.

Intrinsic viscosity values can be directly linked to the molecular weight M of the polymer according to Equation 3.10. For native waxy maize starch, the constant values in this equation are reported to be 0.29 ± 0.04 for the a constant and $0.28 \pm$

1.2 for the K' constant (Millard et al., 1997). Hence, according to these authors, $[\eta]=0.59.M^{0.29}$ which is equivalent to $M=([\eta]/0.59)^{1/0.29}$. This estimation of the starch molecular weight can be plotted versus the NaCl concentration present in the sample during the heat treatment, as represented in Figure 7.11.

4 fold the starch molecular weight.

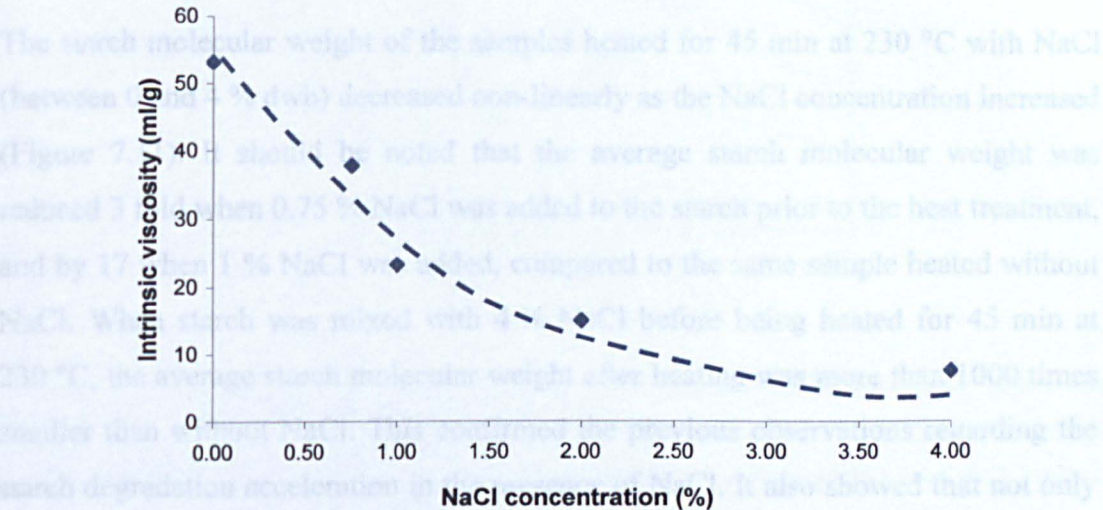


Figure 7.10: Intrinsic viscosity of native waxy maize starch heated at 230 °C for 45 min with different NaCl concentrations (between 0 % and 4 % dwb), dissolved in KOH 5 M and further diluted in distilled water, as a function of the NaCl concentration (% dwb).

All the methods used demonstrated that heating native starches for 45 min at 230 °C tend to enhance the breakdown of the crystallites and the starch molecules. Presence of NaCl with starches during this heat treatment enhanced the starch degradation. The pH of the starches decreased as the heating time increased, which could favour the starch degradation (p-reconversion).

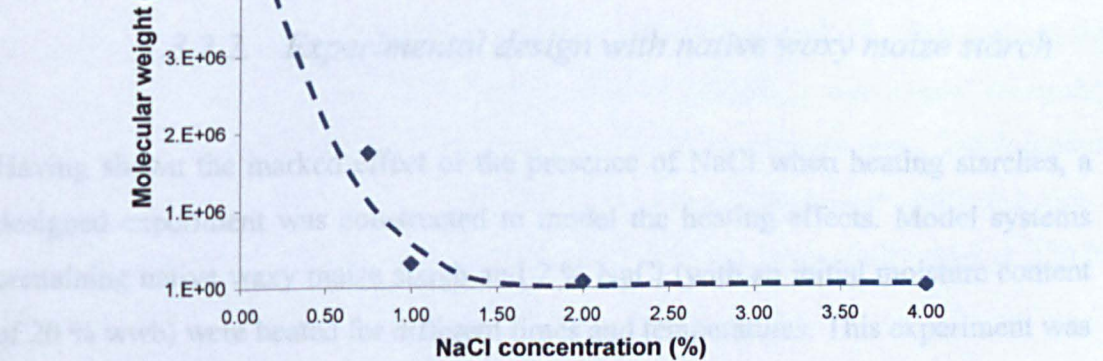


Figure 7.11: Molecular weight of native waxy maize starch heated at 230 °C for 45 min with different NaCl concentrations (between 0 % and 4 % dwb), according to the equation $[\eta]=0.59.M^{0.29}$.

The molecular weight of the non-heated native waxy maize starch dissolved in KOH (5 M) for 2.5 hours was estimated to be around 1.9×10^7 g/mol. When the native waxy maize starch was heated for 45 min at 230 °C, the estimated starch molecular weight decreased to a value of 5.4×10^6 g/mol. Hence, the heat treatment itself decreased 3 to 4 fold the starch molecular weight.

The starch molecular weight of the samples heated for 45 min at 230 °C with NaCl (between 0 and 4 % dwb) decreased non-linearly as the NaCl concentration increased (Figure 7.11). It should be noted that the average starch molecular weight was reduced 3 fold when 0.75 % NaCl was added to the starch prior to the heat treatment, and by 17 when 1 % NaCl was added, compared to the same sample heated without NaCl. When starch was mixed with 4 % NaCl before being heated for 45 min at 230 °C, the average starch molecular weight after heating was more than 1000 times smaller than without NaCl. This confirmed the previous observations regarding the starch degradation acceleration in the presence of NaCl. It also showed that not only the starch granule integrity and crystallinity was affected more efficiently during the heat treatment in the presence of NaCl, but also the starch polymers in themselves.

All the methods used demonstrated that heating native starches for 45 min at 230 °C tend to enhance the breakdown of the crystallites and the starch molecules. Presence of NaCl with the starches during this heat treatment enhanced the starch degradation. The pH of the systems decreased as the heating time increased, which could favour the starch granule degradation (pyroconversion).

7.3.2.2. Experimental design with native waxy maize starch

Having shown the marked effect of the presence of NaCl when heating starches, a designed experiment was constructed to model the heating effects. Model systems containing native waxy maize starch and 2 % NaCl (with an initial moisture content of 20 % ww) were heated for different times and temperatures. This experiment was done in order to observe the influence of these 2 parameters on colour formation (measured according to section 3.2.2.2) and native starch granule degradation.

Colour formation of these model systems after a heat treatment between 15 and 90 min at temperatures between 180 and 230 °C, is presented in Figure 7.12.

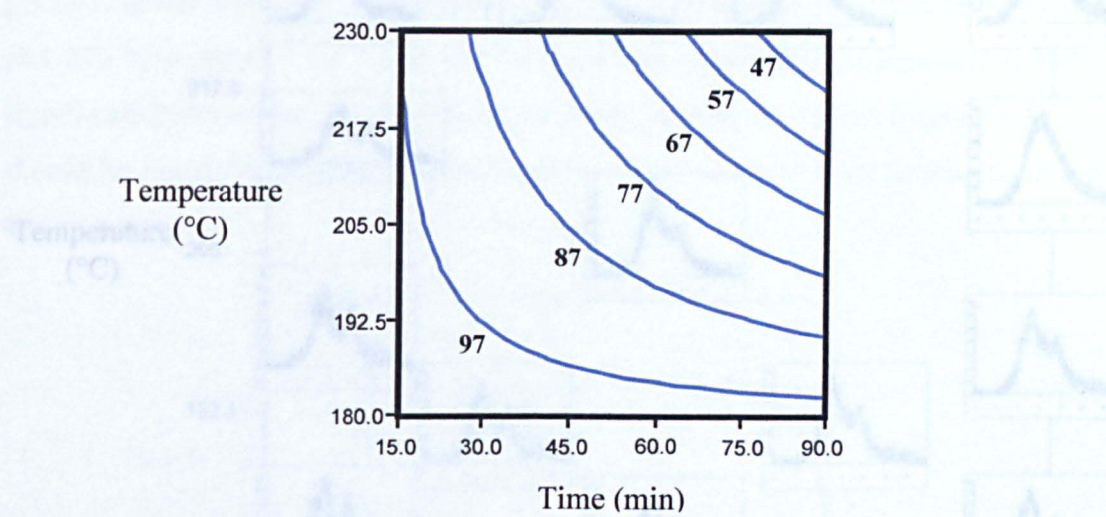


Figure 7.12: L* value (as annotated on the graph) of model systems containing native waxy maize starch and 2 % of NaCl (dwb), with a moisture content of 20 % (wwb), heated between 15 and 90 min at temperatures between 180 °C and 230 °C . Corresponding equation: L^* value = $76.04 + 2.79 * \text{Time} + 0.14 * \text{Temperature} - 0.02 * \text{Time} * \text{Temperature}$. $r^2 = 0.90$.

Heating time and temperature both had a significant influence on colour formation of these models ($p < 0.05$) when the results were analysed using Design Expert (see section 3.2.4). Increasing these parameters led to darker products. The pH of the model system suspensions in water followed the same trend as the colour formation: it decreased as the product darkness increased (data not shown). This might be due to the caramelisation reactions leading to both colour and H^+ formation.

X-ray measurements of these samples are presented in Figure 7.13. They showed that within this range of temperatures, starch granule crystallinity was altered when they were heated for more than 15 min, regardless of the temperature used. Starch granule crystallinity loss was less effective when lower temperatures were applied; hence it took longer times at 180 °C to alter the starch granule crystallinity than at 230 °C (Figure 7.13).

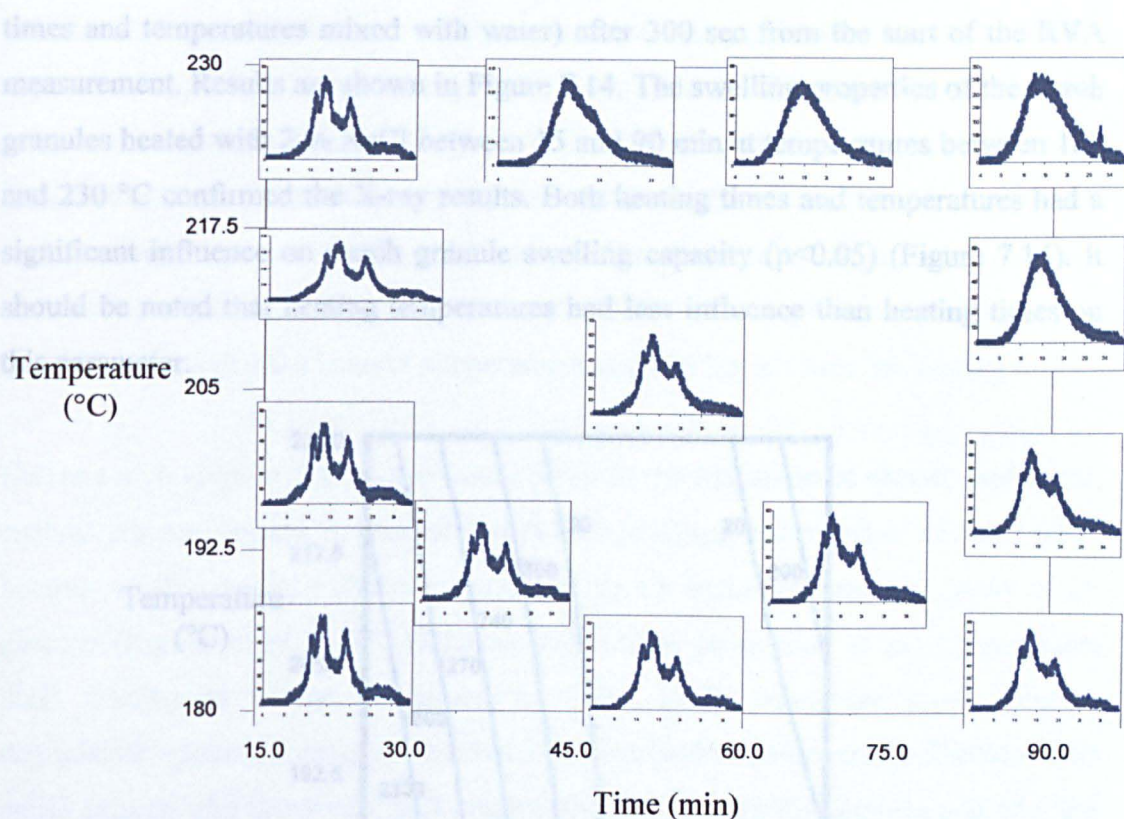


Figure 7.13: Wide angle X-ray patterns of native waxy maize starch mixed with 2 % NaCl (dwb) heated between 15 and 90 min at temperatures between 180 and 230 °C; measurements performed at angles between 4 and 38 ° (2 θ) with a step size of 0.02 ° and a time per step of 3 s.

The starch granule integrity was also evaluated by measuring the swelling capacities of the heat treated starches and their aptitude to increase a solution viscosity, as measured with an RVA. In order to evaluate the influence of heating times and temperatures on the starch swelling properties of the granules, one data point in the viscosity pattern obtained for each sample was chosen. The viscosity of a suspension of native waxy maize starch (4.00 g dwb) heated in distilled water (total suspension weight of 28.00 g) at 14 °C/min from 25 °C to 95 °C reached a maximum viscosity after 300 sec, which corresponded to a temperature around 80 °C (data not shown). At that stage, the waxy maize starch granules were gelatinised, hence they were swollen. However, as the pasting times were still short, only few starch molecules leached out of the granule, which would cause a decrease of the solution viscosity with further heating.

It was chosen to take into account in the experimental design the viscosity of the model system suspension (waxy maize starch with 2 % NaCl, heated for different

times and temperatures mixed with water) after 300 sec from the start of the RVA measurement. Results are shown in Figure 7.14. The swelling properties of the starch granules heated with 2 % NaCl between 15 and 90 min at temperatures between 180 and 230 °C confirmed the X-ray results. Both heating times and temperatures had a significant influence on starch granule swelling capacity ($p < 0.05$) (Figure 7.14). It should be noted that heating temperatures had less influence than heating times on this parameter.

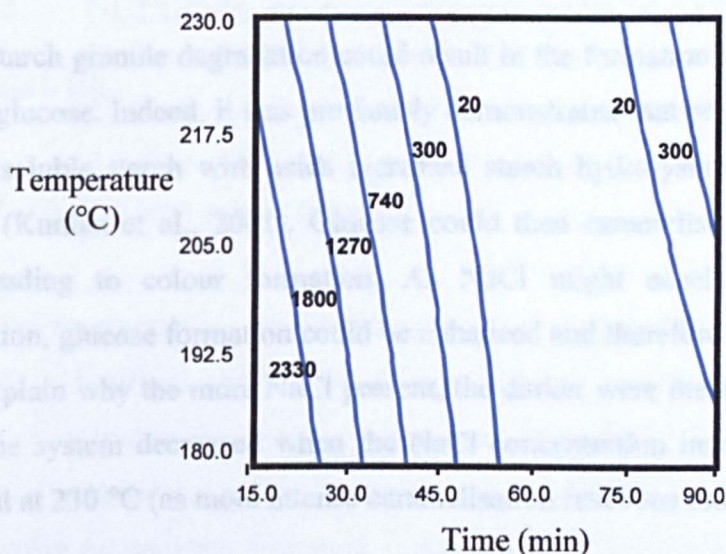


Figure 7.14: Influence of heating time (from 15 to 90 min) and heating temperature (from 180 to 230 °C) on the starch granule swelling properties of models composed of native waxy maize starch mixed with 2 % NaCl (dwb). The data annotated on the graph correspond to the solution viscosity (cP) of 4.00 g of model system (dwb) adjusted to 28.00 g with distilled water and heated for 300 sec at 14 °C/min from an initial temperature of 25 °C. Solution viscosity = $10821 - 233 * \text{Time} - 34 * \text{Temperature} + 0.91 * \text{Time}^2 + 0.54 * \text{Time} * \text{Temperature}$. $r^2 = 0.93$.

Solution viscosity (observed with the RVA measurements) started to increase again when the model systems were heated for the highest heating times and temperatures. This might be due to some polymerisation of the molecules present in the systems due to the severe heat treatment, as shown in the literature review (Figure 2.19). Indeed, the samples heated for more than 75 min at 230 °C were harder to mix with distilled water.

From the set of experiments used in this study (sections 7.3.2.1 and 7.3.2.2), it was observed that the presence of NaCl with native starch during a heat treatment

enhanced colour formation of the samples, proportionally to the NaCl's concentration. Microscopic observations, X-ray, RVA, DSC and intrinsic viscosity measurements all suggested that even without NaCl, native starch granules were degraded by such a heat treatment. However, when NaCl was added, this degradation was accelerated proportionally to its amount. Starch granules' degradation occurred even at lower temperatures (180 °C), but then longer heating times were required. It should be noted that the heating temperatures had less impact than the heating times.

Native starch granule degradation could result in the formation of shorter molecules, such as glucose. Indeed, it was previously demonstrated that presence of NaCl when heating soluble starch with acids increased starch hydrolysis and the yield of D-glucose (Kunlan et al., 2001). Glucose could then caramelize at the temperatures used, leading to colour formation. As NaCl might accelerate starch granule degradation, glucose formation could be enhanced and therefore caramelisation. This could explain why the more NaCl present, the darker were the samples and why the pH of the system decreased when the NaCl concentration increased during a heat treatment at 230 °C (as more intense caramelisation reactions took place).

For breakfast cereal products, toasting temperatures between 180 and 235 °C are used. It was observed with the model systems that even at 180 °C, starch granule alteration occurred, but longer heating times were needed. Colour measurement of the wheat and rice mixture, corn and rice breakfast cereals demonstrated that presence of NaCl in the recipe led to darker products, even for cereals toasted at 180, 190 and 200 °C (Table 5.5). Hence, alteration of the starch polymer could be envisaged in breakfast cereals even for the low toasting temperatures. It should be kept in mind that the models composed of native starch and NaCl were not directly applicable as such to determine possible starch degradation in breakfast cereal products, but give only trends of the impact of heating times and temperatures. Therefore, the significant influence of NaCl on colour formation of breakfast cereals at a temperature of 180 °C (contrary to the model system where it took longer times) could be explained by the differences in terms of composition between the food product and the model system. It could also be due to the differences in terms of heat treatment between the oven used for the model and the one used for the food product.

The starch present in breakfast cereals was not in the form of a native granules, but was gelatinised. To confirm whether starch alteration occurred during a heat treatment, even for starch molecules not constrained in the form of a granule, the influence of NaCl on pregelatinised starch during toasting was observed. Results are presented in the following section.

7.4. Influence of NaCl on pregelatinised starch during toasting

7.4.1. System preparation

Model systems containing pregelatinised waxy maize starch and NaCl (0.00, 0.75, 1.00, 2.00 and 4.00 % NaCl dwb), with a moisture content of 15 % (wwb) were prepared according to the procedure explained in section 3.2.1.2. Aliquots of 45 g were placed in open aluminium containers and were heated in an oven for 40 min at 230 °C. After the toasting treatment, these models were dissolved in KOH 1 M at concentrations of 20 mg of starch/g of solution. NaCl was added in each solution to have the same NaCl concentration as the sample of pregelatinised starch heated in the presence of 4 % NaCl and dissolved in KOH (1 M). These solutions were gently stirred for 16 h to ensure complete dissolution of the starchy sample. All measurements were performed within 1 h in a random order to be able to compare the data taking into account possible starch degradation by KOH.

7.4.2. Results and discussion

It was previously observed that NaCl seemed to accelerate native starch granule breakdown into smaller molecules during a heat treatment, which could explain the impact of NaCl on colour formation. If NaCl also influences colour formation of pregelatinised starch samples while heating, an eventual starch breakdown should also be considered. Therefore, models containing waxy pregelatinised starch and NaCl (0 to 4 % dwb) with an initial moisture content of 15 % (wwb) were heated in

an oven for 40 min at 230 °C. The influence of NaCl concentration on colour formation is represented in Figure 7.15. Colour formation followed the same trend as that observed for models containing native starch and NaCl and all the other models used previously that contained starch, glucose and amino-acids. NaCl concentration increased the darkness of the models proportionally to its concentration (linear correlation, $r^2 = 0.91$) (Figure 7.15). As a negligible amount of protein was present in the system, colour formation could be attributed mainly to caramelisation reactions.

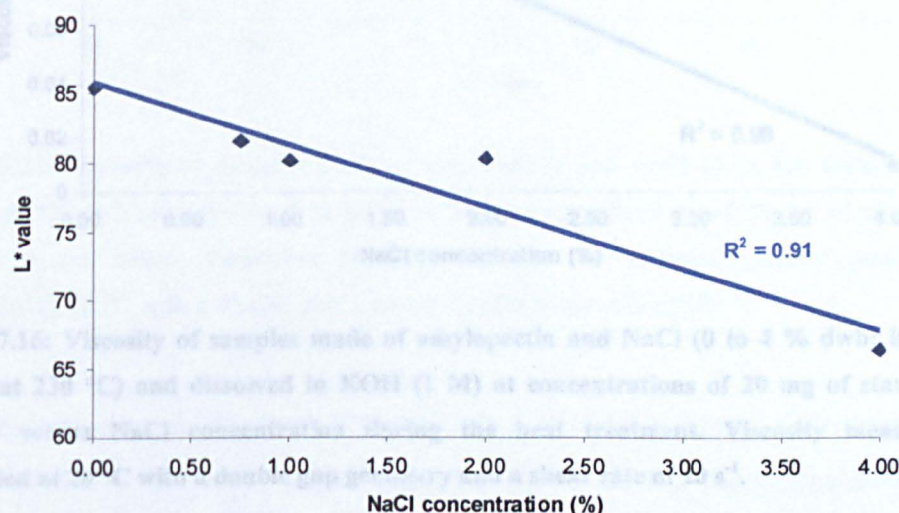


Figure 7.15: Influence of NaCl concentration (% dwb) on the L* value of pregelatinised waxy maize starch and NaCl heated at 230 °C for 40 min in an open container.

The viscosity of a polymer solution in a solvent is influenced by several polymer characteristics: its concentration, conformation and molecular weight (Lapasin and Prici, 1995). To check whether NaCl enhanced starch degradation, the samples of heated waxy pregelatinised starch with NaCl (0 to 4 %) were dissolved in KOH (1 M) to have the same final starch concentrations in each solution. The amount of NaCl was adjusted to have also the same NaCl concentration in each solution. If it is assumed that the amylopectin conformation is similar regardless of the NaCl concentration of the sample during the heat treatment, the viscosity of these solutions should be correlated to the average molecular weight of amylopectin as its concentration was the same in each solution. The viscosity was measured at 20 °C for shear rates between 10 s⁻¹ and 25 s⁻¹ (see section 3.2.2.6). The solution viscosity,

at 20 s^{-1} , versus the NaCl concentration present in the starch sample during the heat treatment at $230\text{ }^{\circ}\text{C}$, is shown in Figure 7.16.

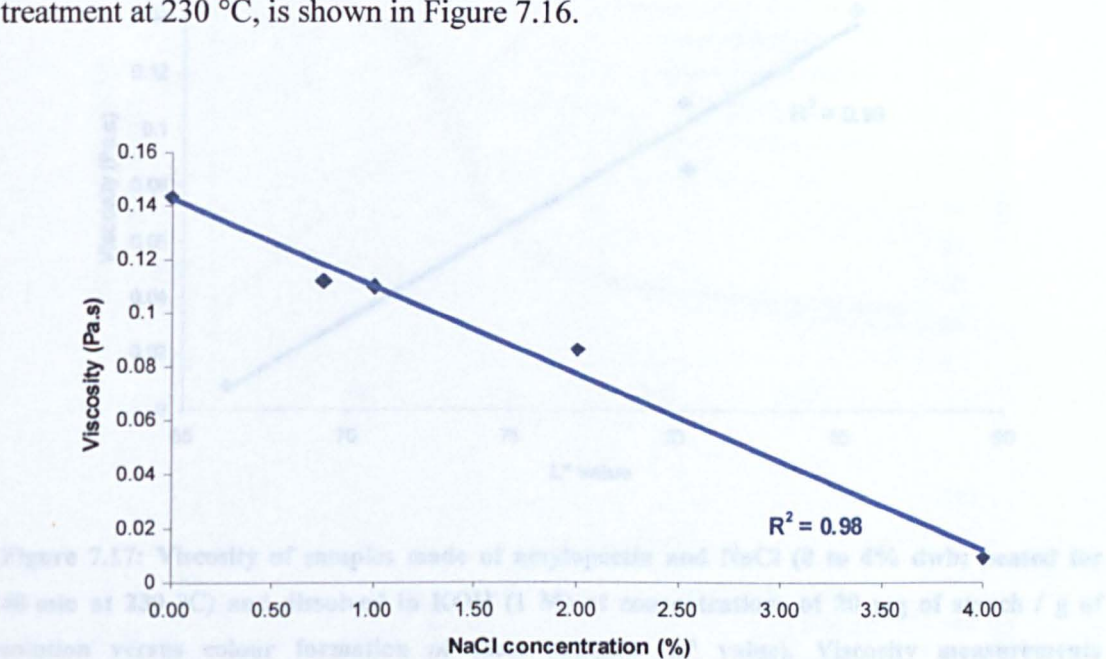


Figure 7.16: Viscosity of samples made of amylopectin and NaCl (0 to 4 % dwb; heated for 40 min at $230\text{ }^{\circ}\text{C}$) and dissolved in KOH (1 M) at concentrations of 20 mg of starch / g of solution versus NaCl concentration during the heat treatment. Viscosity measurements performed at $20\text{ }^{\circ}\text{C}$ with a double gap geometry and a shear rate of 20 s^{-1} .

Linear correlations between the sample colour and the sample solution viscosity were observed with a regression coefficient range between 0.79 to 0.98, depending on the shear rate. The linear correlation between the NaCl concentration in the samples during the heat treatment at $230\text{ }^{\circ}\text{C}$ and the viscosity of the corresponding sample dissolved in KOH showed that the higher was the NaCl concentration during a heat treatment, the smaller might be the molecular weight of the resulting polymer. This result seems to corroborate the previous data obtained with the intrinsic viscosity measurements of the samples containing native starch.

As a linear correlation was previously observed between NaCl concentration during the heat treatment and colour formation, a linear relationship was also observed between the viscosity and the L^* value of the sample (Figure 7.17).

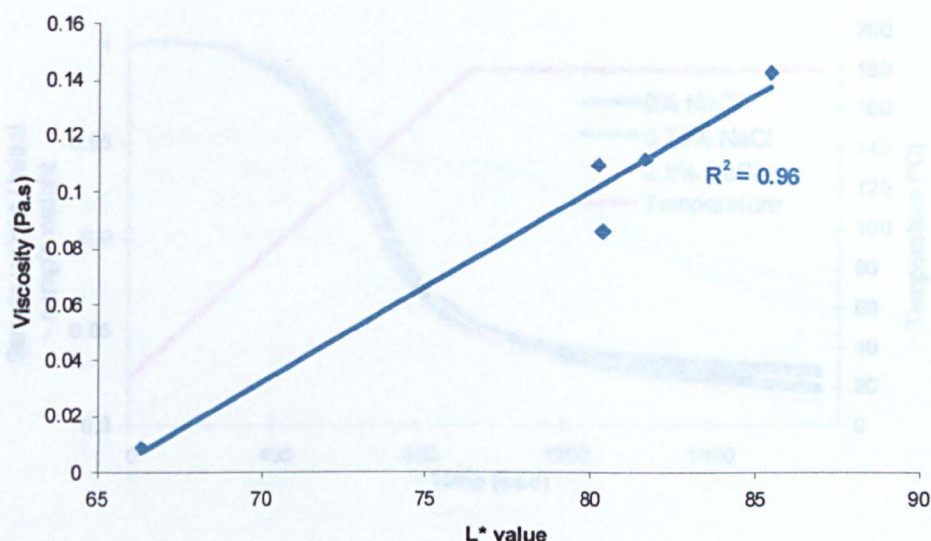


Figure 7.17: Viscosity of samples made of amylopectin and NaCl (0 to 4% dwb; heated for 40 min at 230 °C) and dissolved in KOH (1 M) at concentrations of 20 mg of starch / g of solution versus colour formation of these samples (L* value). Viscosity measurements performed at 20 °C with a double gap geometry and a shear rate of 20 s⁻¹.

The previous experiments seemed to indicate that NaCl enhanced the starch granule and the starch polymer degradation during a severe heat treatment. TGA measurements of breakfast cereal products were performed to check whether such starch alteration could be observed in food products by following their weight loss during heating. Indeed, if starch polymers were altered by the heat treatment, the resulting compounds (such as glucose) could react further and generate volatile molecules leading to a sample weight loss.

Wheat, corn and rice doughs (around 20 % moisture) were heated either from 25 °C to 180 °C at 10 °C/min and were held at 180 °C for 10 min or they were heated from 25 °C to 250 °C at 10 °C/min and were held at 250 °C for 10 min. The weight of the sample was recorded during this heat treatment. Results are shown in Figure 7.18 and Figure 7.19.

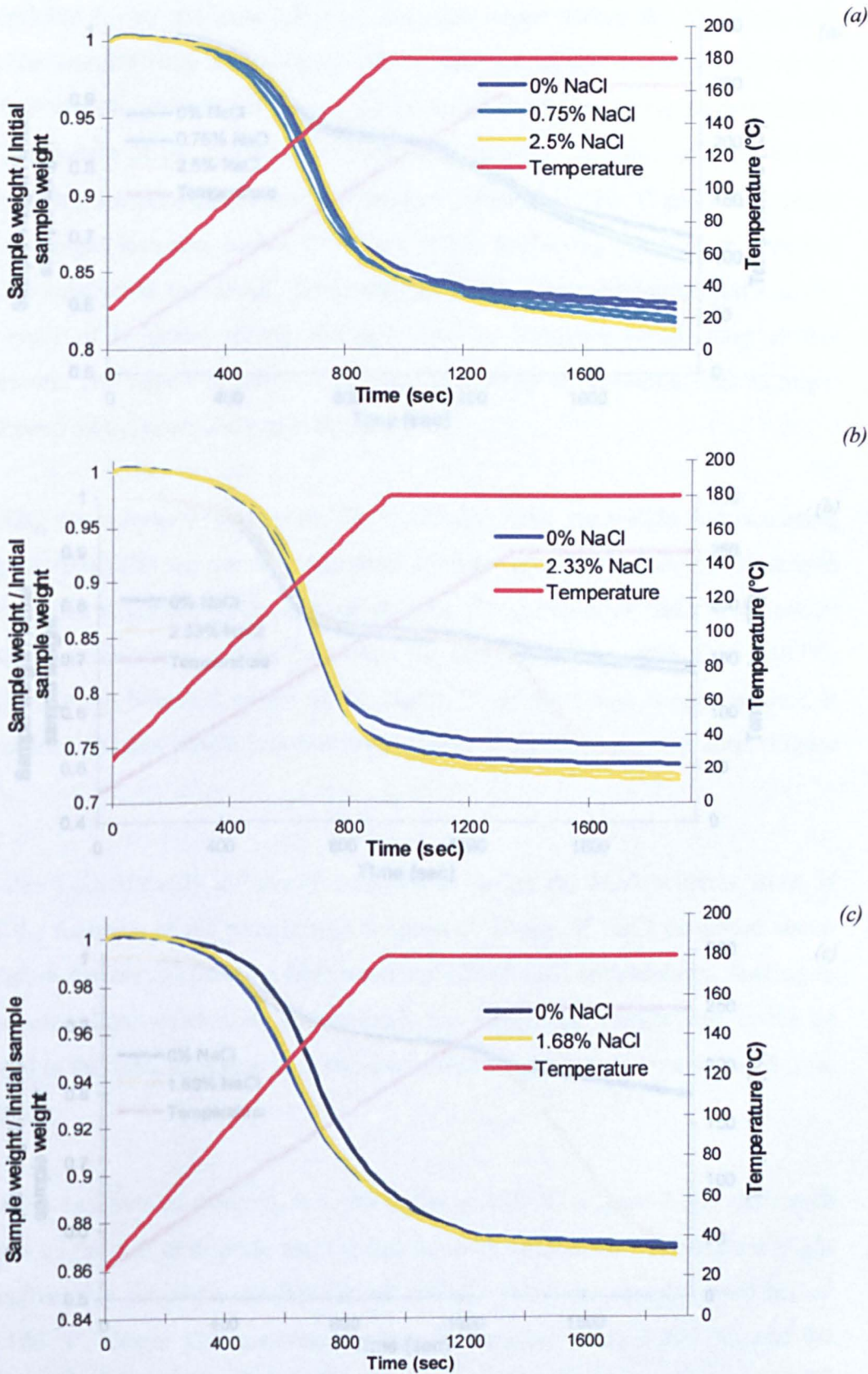


Figure 7.18: TGA analyses of wheat (a), corn (b) and rice (c) breakfast cereal doughs heated from 25 °C to 180 °C at 10 °C/min and held at 180 °C for 10 min. Samples measured and presented in duplicates.

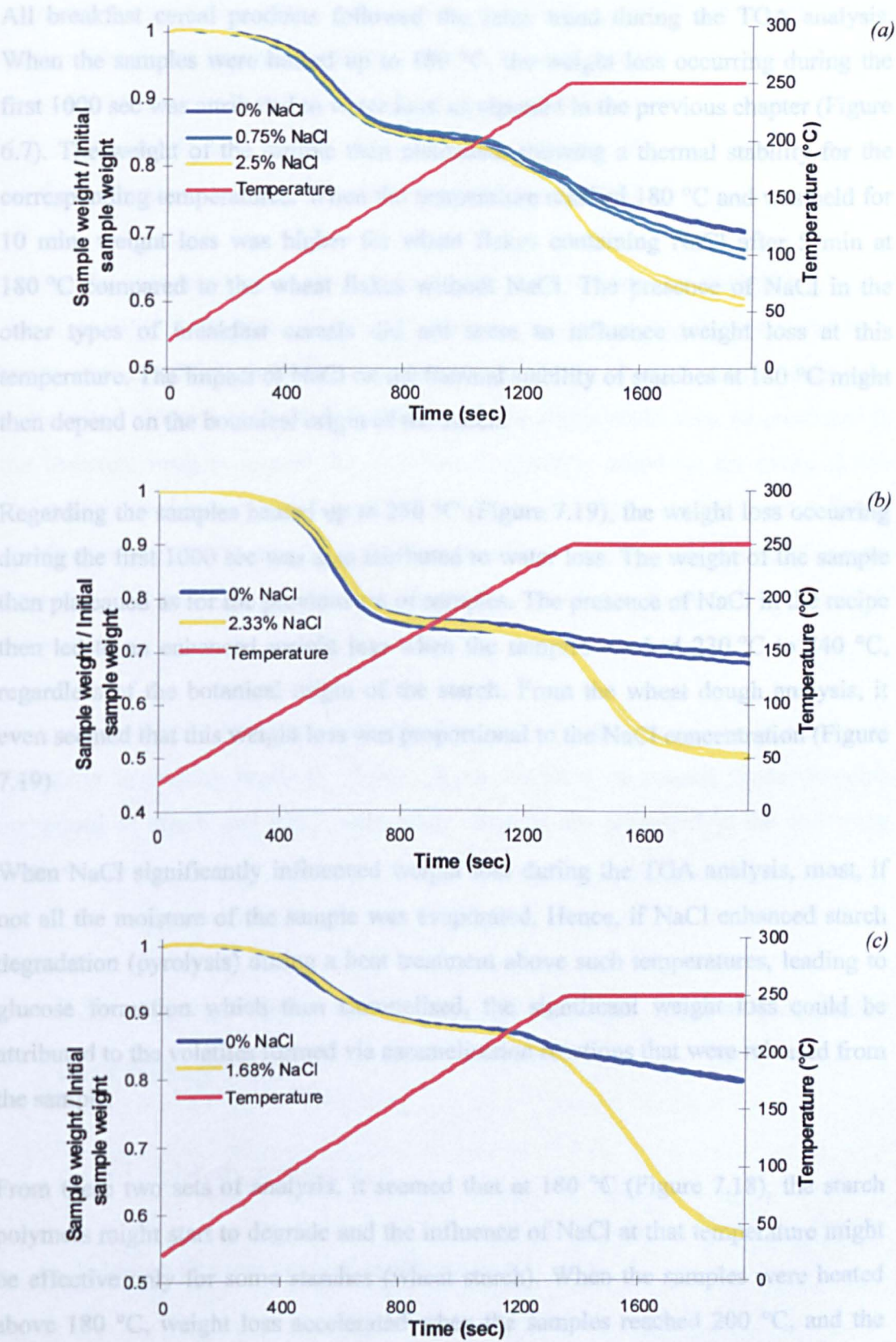


Figure 7.19: TGA analyses of wheat (a), corn (b) and rice (c) breakfast cereal doughs heated from 25 °C to 250 °C at 10 °C/min and held at 250 °C for 10 min. Samples measured and presented in duplicates.

All breakfast cereal products followed the same trend during the TGA analysis. When the samples were heated up to 180 °C, the weight loss occurring during the first 1000 sec was attributed to water loss, as reported in the previous chapter (Figure 6.7). The weight of the sample then plateaued, showing a thermal stability for the corresponding temperatures. When the temperature reached 180 °C and was held for 10 min, weight loss was higher for wheat flakes containing NaCl after 5 min at 180 °C compared to the wheat flakes without NaCl. The presence of NaCl in the other types of breakfast cereals did not seem to influence weight loss at this temperature. The impact of NaCl on the thermal stability of starches at 180 °C might then depend on the botanical origin of the starch.

Regarding the samples heated up to 250 °C (Figure 7.19), the weight loss occurring during the first 1000 sec was also attributed to water loss. The weight of the sample then plateaued as for the previous set of samples. The presence of NaCl in the recipe then led to an enhanced weight loss when the samples reached 230 °C to 240 °C, regardless of the botanical origin of the starch. From the wheat dough analysis, it even seemed that this weight loss was proportional to the NaCl concentration (Figure 7.19).

When NaCl significantly influenced weight loss during the TGA analysis, most, if not all the moisture of the sample was evaporated. Hence, if NaCl enhanced starch degradation (pyrolysis) during a heat treatment above such temperatures, leading to glucose formation which then caramelised, the significant weight loss could be attributed to the volatiles formed via caramelisation reactions that were released from the sample.

From these two sets of analysis, it seemed that at 180 °C (Figure 7.18), the starch polymers might start to degrade and the influence of NaCl at that temperature might be effective only for some starches (wheat starch). When the samples were heated above 180 °C, weight loss accelerated when the samples reached 200 °C, and the influence of NaCl on this weight loss became noticeable for temperatures of 230 °C and above, regardless of the type of starch. From the previous results, it was observed that toasting temperatures of 180 °C led to lighter wheat and rice mixture breakfast cereal products when they did not contain NaCl (Table 5.5). This

observation corroborates the results presented here, that 180 °C was a high enough temperature to see an impact of NaCl on wheat based products alteration. Rice and corn breakfast cereals had a toasting temperature above 200 °C. Hence, starch degradation might also be effective during the process of these two products (as confirmed by the colour measurements presented in Table 5.5).

The model systems used so far in this chapter were prepared by suspending native starch granules or dissolving starch molecules (in the case of pregelatinised starch) in water, and dissolving some NaCl in this suspension or solution. At that stage, the sodium and chloride ions might interact with the starch molecules, as presented in the literature review (section 2.3.2.2). This interaction might be the cause of the different thermal behaviour of the starches as was reported by several authors. Bryce and Greenwood (1963) showed that presence of NaCl with amylo maize starch heated between 220 and 340 °C in high vacuum lowered the threshold of pyrolysis temperature.

Such starch / NaCl interactions could have also an influence on the NaCl crystal formation in starchy products. Hence, X-ray analyses of several model systems composed of starch and NaCl were made. Results are presented in the following section.

7.5. X-ray measurements of starch based products containing NaCl

7.5.1. System preparation

- Models with pregelatinised starch and NaCl

Model systems composed of pregelatinised starch and NaCl (concentrations between 5 and 15 % dwb) were mixed with water to obtain a homogeneous paste. This paste was frozen and freeze dried. The models were then milled and sieved (< 250 µm). The resulting powders were stored over P₂O₅ or saturated salt solutions of

CH₃COOK, K₂CO₃ and NaNO₂ which gave water activity values of 0, 0.23, 0.43 and 0.64 respectively (Biliaderis et al., 1999). The detailed procedure was presented in section 3.2.1.6.

- Breakfast cereal products

Wheat and rice mixture, corn and rice breakfast cereals containing different levels of NaCl were used in this study. Their preparation was explained in section 3.2.1.5.

7.5.2. Results and discussion

When NaCl is dry (not dissolved in solution), it is in a crystal form. As explained in Chapter 3, X-rays detect regular arrangements of atoms as occurring in crystals. Hence, X-rays are able to detect NaCl when it is under the shape of a crystal. The angle at which NaCl lead to constructive interferences of the X-ray beams is 32 °.

The wheat, wheat and rice mixture, corn and rice breakfast cereal products with NaCl used in this study contained between 1.32 and 2.50 % of NaCl depending on the recipe. When these breakfast cereals were measured by X-ray, patterns similar to the one presented in Figure 7.20 were obtained for each of these products.

NaCl crystals were not observed for corn flakes containing 2.33 % of NaCl (added at the beginning of the process). No NaCl crystals were ever observed in breakfast cereal products for the NaCl concentrations studied. However, such low NaCl concentrations could be detected, as the addition of 2 % ground NaCl crystals to milled corn flakes (which did not contain NaCl) led to a clear peak for an angle of 32 ° (Figure 7.20). To observe whether the absence of NaCl crystal peaks in breakfast cereals was due to the presence of starch (and not to other ingredients), model systems composed of pregelatinised starch and NaCl were analysed by X-ray (Figure 7.21). In model systems composed of pregelatinised starch and NaCl with a moisture content approaching 0 % (Figure 7.21), concentrations of 10 % or less NaCl did not lead to detectable crystal formation. This confirmed that it was probably the

presence of starch in breakfast cereals which prevented NaCl crystal formation, as it was the case in these model systems.

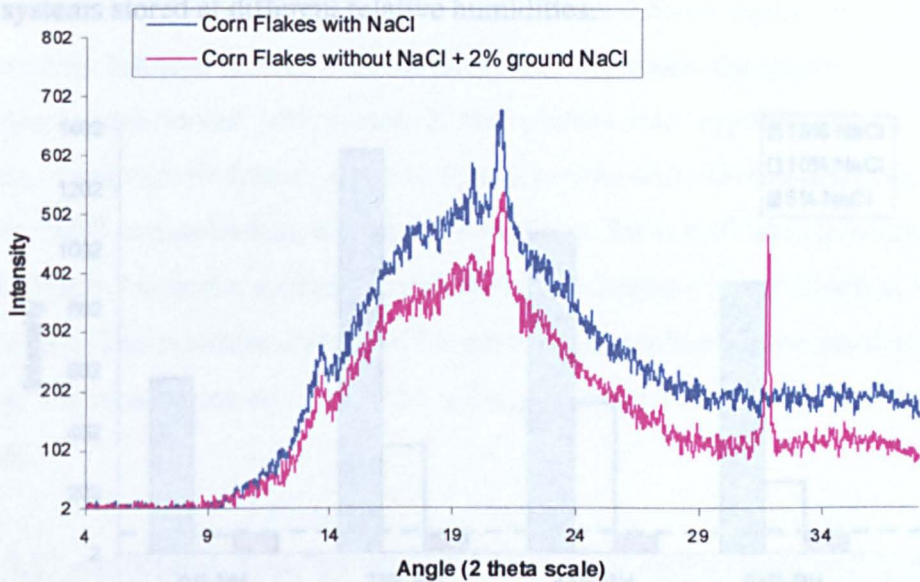


Figure 7.20: Wide angle X-ray patterns of corn flakes produced with 2.33 % NaCl and corn flakes produced without NaCl, milled and with 2 % ground NaCl added ; measurements performed at angles between 4 and 38 ° (2θ) with a step size of 0.02 ° and a time per step of 3 s.

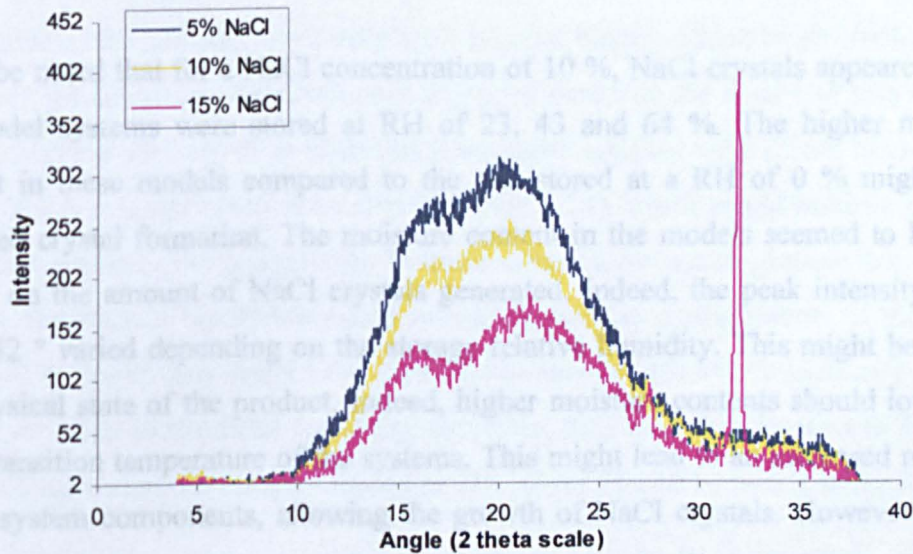


Figure 7.21: Wide angle X-ray patterns of model systems composed of pregelatinised starch and NaCl (between 5 and 15 % dwb) stored for 3 weeks at a relative humidity of 0 %; measurements performed at angles between 4 and 38 ° (2θ) with a step size of 0.02 ° and a time per step of 3 s.

When these model systems were stored at other relative humidities, NaCl crystals were observed for NaCl concentrations of 10 % (dwb) and above. Figure 7.22 shows the peak intensity for an angle of 32° of wide angle X-ray patterns of the above model systems stored at different relative humidities.

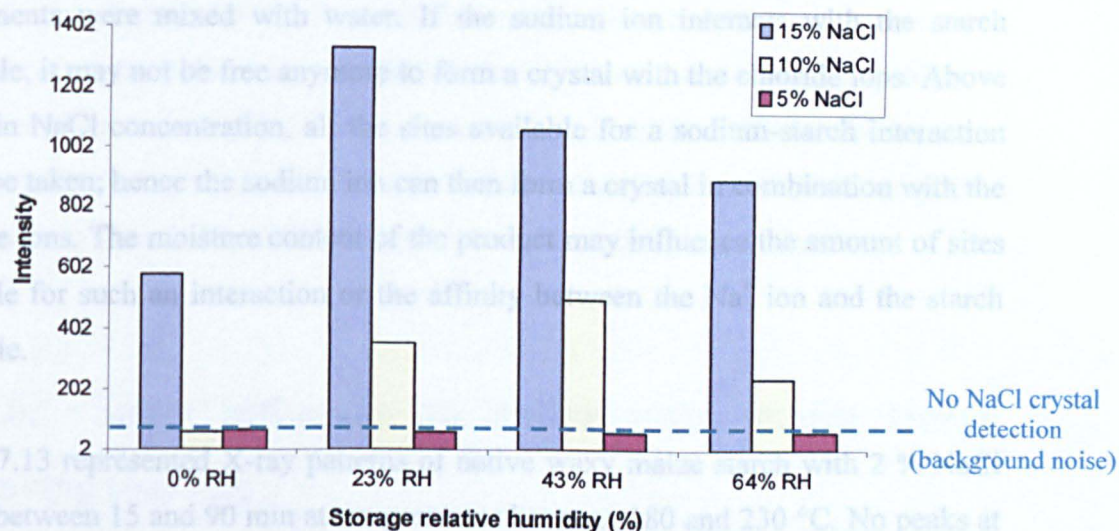


Figure 7.22: Peak intensity at an angle of 32° (2θ) of wide angle X-ray patterns (step size of 0.02° , time per step of 3 s) of model systems composed of pregelatinised starch and NaCl (between 5 and 15 % dwb) stored for 3 weeks at different relative humidities.

It can be noted that for a NaCl concentration of 10 %, NaCl crystals appeared when the model systems were stored at RH of 23, 43 and 64 %. The higher moisture content in these models compared to the one stored at a RH of 0 % might have triggered crystal formation. The moisture content in the models seemed to have an impact on the amount of NaCl crystals generated. Indeed, the peak intensity at the angle 32° varied depending on the storage relative humidity. This might be due to the physical state of the product. Indeed, higher moisture contents should lower the glass transition temperature of the systems. This might lead to an increased mobility of the system components, allowing the growth of NaCl crystals. However, above certain moisture contents, some NaCl might dissolve in the water present, hence decreasing the NaCl crystal amount. At a NaCl concentration of 5 %, no NaCl crystals were detected regardless of the relative humidity at which was stored the model systems. Hence, if sodium chloride has the same behaviour in the models as in breakfast cereals, the NaCl concentration in the food product was not high enough to

get any crystal detection. In the literature review, it was mentioned that several authors found that electrolytes, such as sodium and chloride ions, can interact with starch granules in an aqueous solution (section 2.3.2.2). The formation of alcoholate was even mentioned (Oosten, 1983). These interactions could explain the absence of NaCl crystals below a certain concentration, as, to prepare the model systems, the components were mixed with water. If the sodium ion interacts with the starch molecule, it may not be free anymore to form a crystal with the chloride ions. Above a certain NaCl concentration, all the sites available for a sodium-starch interaction might be taken; hence the sodium ion can then form a crystal in combination with the chloride ions. The moisture content of the product may influence the amount of sites available for such an interaction or the affinity between the Na^+ ion and the starch molecule.

Figure 7.13 represented X-ray patterns of native waxy maize starch with 2 % NaCl heated between 15 and 90 min at temperatures between 180 and 230 °C. No peaks at the angle of 32 ° could be observed for almost all the patterns, which demonstrated that the interactions between the electrolytes and the starch molecules might occur even when the starch was under the form of a granule. However, a peak was detected when the model systems were heated at 230 °C for 71 and 90 min. At these temperatures, the samples were very dark (almost black), which might indicate that the starch granules were tremendously degraded based on the results of this chapter. A viscosity increase was even observed when these samples were mixed in water and heated (RVA measurements presented in Figure 7.14), which might indicate possible repolymerisation of the compounds. In these samples, the starch granule degradation and the compounds repolymerisation might change the configuration of the starch molecule. It might then disable the interactions between the starch molecule and the electrolytes. The electrolytes becoming available, they may then interact to form the crystals observed in the samples heated at 230 °C for 71 and 90 min (Figure 7.13).

The interactions between the sodium and chloride ions and the starch granules, while the granules were in an aqueous suspension, might change the starch granule properties. Indeed, after the drying step of the suspension to a moisture content of 20 % (wwb), the starch granule behaviour during the heat treatment at 230 °C might be modified. Its interactions with NaCl might then explain the starch degradation

enhancement, which was proportional to the NaCl concentration. Other types of salts might also interact with the starch granules and enhance the starch degradation during a heat treatment at 230 °C. It was previously reported that binding copper ions to wheat and potato native starch granules changed the thermal stability of the granules. A significant increase of the starch decomposition enthalpy accompanied by a decrease of about 20 °C of the starch decomposition temperature was observed (Szymonska et al., 2008). Lai et al. (2001) also observed that the thermal stability of the soaked starches in MgCl_2 (0.5 M) decreased to a level depending on both cations and anions remaining in granule. Therefore, the next section focuses on the impact of several salts on starch degradation to understand the underlying mechanism.

7.6. Salt's influence on native waxy maize starch during toasting

7.6.1. System preparation

Model systems containing native waxy maize starch and one type of salt (NaCl, KCl, CaCl_2 , LiCl, MgCl_2 , NaI, KNO_3 , NaBr, KBr, Na_2SO_4 , NaNO_3 , Na_2CO_3 or KI) for a concentration of 0.030 mol of salt / 100 g of dry starch were made following the same procedure as the models containing native starch and NaCl (see section 3.2.1.1). The moisture content of these models was 20 % (wwb). Aliquots of 50 g were heated for 30 min at 230 °C.

Model systems containing 0.015, 0.020 and 0.025 mol of CaCl_2 or MgCl_2 / 100 g of dry starch were also prepared in the same way.

7.6.2. Results and discussion

The previous study concluded that in model systems composed of native or pregelatinised starch mixed with NaCl between 0 and 4 % (dwb), NaCl significantly enhanced colour formation proportionally to its concentration when these systems

were heated above 180 °C in open containers. Microscopic observations, wide angle X-ray, RVA, intrinsic viscosity and DSC data all suggested that NaCl accelerated the starch granules and the starch polymer’s degradation during the heat treatment. This toasting treatment potentially formed smaller molecules like glucose, in a similar way as during pyroconversion. The glucose that may be formed could then caramelise during the heat treatment which might explain the impact of sodium chloride on colour formation. In order to understand better the mechanism by which NaCl enhanced colour formation and starch degradation during such a heat treatment, this study aimed at investigate the influence of several types of salts on starch granule loss of crystallinity (hence starch granule alteration).

Twelve types of salts (MgCl₂, CaCl₂, LiCl, Na₂SO₄, NaCl, NaBr, NaNO₃, NaI, KCl, KNO₃, KBr or KI) were mixed with waxy maize starch (same salt/starch molar ratio in each sample) and were heated at 230 °C for 30 min. One sample without salt was used as a control. The colour (L* value) of each sample is shown in Figure 7.23. A statistical analysis was performed regarding the salt influence on the L* value of the samples.

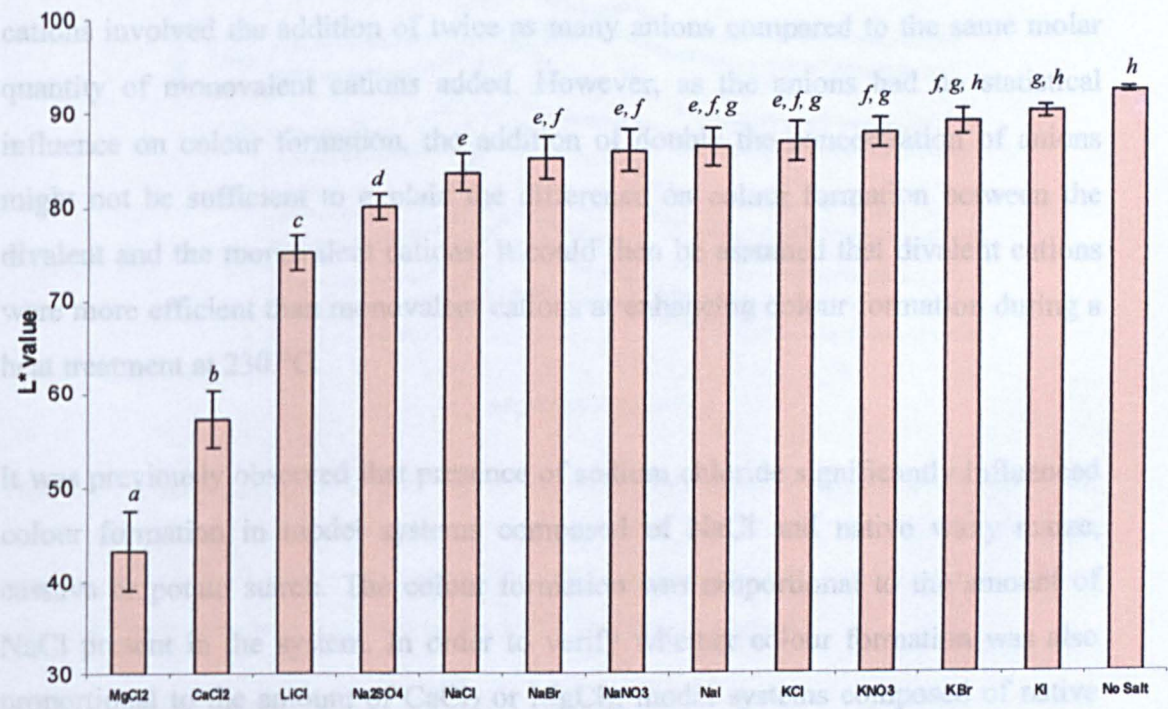


Figure 7.23: L* value of starch samples mixed with different types of salt and heated at 230 °C for 30 min. Samples made in triplicate and colour measurements also performed in triplicate. The letters above each bar indicate significant differences between the samples (p<0.05).

All salts used in this study significantly decreased the L^* values of the waxy maize samples after heating, except KI and KBr which were not significantly different from the no salt control.

As the salt's molar concentration was the same in each sample, it was possible to classify the anions and cations regarding their influence on colour formation. From the darker to the lighter samples, the following classifications can be made: for the divalent cations, $Mg^{2+} > Ca^{2+}$ and for the monovalent cations, $Li^+ > Na^+ > K^+$. The same ordering was obtained regardless of the anion with which they were complexed (Cl^- , Br^- , NO_3^- and I^-). No statistical differences were found regarding the influence of anions on colour formation, neither among the sodium salts, nor among the potassium salts.

Hence, according to the statistical analysis, cations had a stronger impact on colour formation than anions as changing the cation in the salt led to significantly different colour formation ($p < 0.05$), while changing the anion led to equivalent colour formation ($p > 0.05$). It was not possible to compare directly monovalent and divalent cations for their influence on colour formation. Indeed, the addition of divalent cations involved the addition of twice as many anions compared to the same molar quantity of monovalent cations added. However, as the anions had no statistical influence on colour formation, the addition of double the concentration of anions might not be sufficient to explain the difference on colour formation between the divalent and the monovalent cations. It could then be assumed that divalent cations were more efficient than monovalent cations at enhancing colour formation during a heat treatment at 230 °C.

It was previously observed that presence of sodium chloride significantly influenced colour formation in model systems composed of NaCl and native waxy maize, cassava or potato starch. The colour formation was proportional to the amount of NaCl present in the system. In order to verify whether colour formation was also proportional to the amount of $CaCl_2$ or $MgCl_2$, model systems composed of native waxy maize starch and one of these salts (concentration between 0.015 and 0.025 mol / 100 g of dry starch) were made. The models were then heated for 30 min

at 230 °C. It was observed that the darkness of the models increased proportionally with the amount of CaCl_2 or MgCl_2 present (data not shown).

It was also previously presented that the colour formation of model systems containing NaCl and native starch heated at 230 °C was positively correlated to the starch granule loss of crystallinity (as measured by X-ray, data presented in Figure 7.6). To observe whether the influence of the several salts used in this study on colour formation was also linked to the starch crystallinity, the heated samples were analysed by wide angle X-ray (Figure 7.24). To evaluate starch crystallinity, ratios between the area under the peaks and the total area under the curve were measured (see section 3.2.2.9). A linear correlation between the starch crystallinity of the samples and the colour formation can be observed as shown in Figure 7.25.

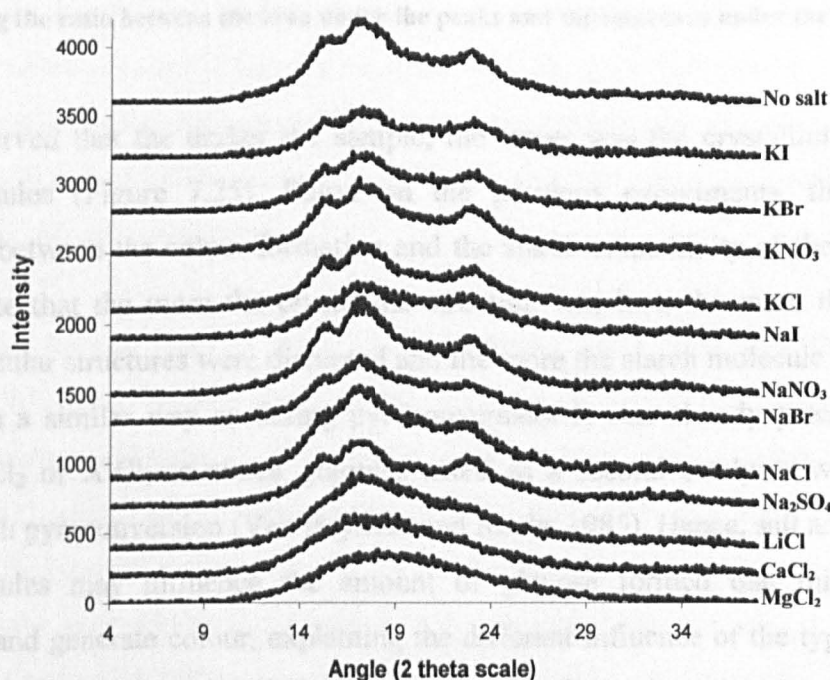


Figure 7.24: Wide angle X-ray patterns of starch samples mixed with different types of salt (0.030 mol of salt / 100 g of dry starch) and heated at 230 °C for 30 min. Measurements performed at angles between 4 and 38 ° (2 θ), with a step size of 0.05 ° and a time per step of 1.5 s, the curves have been displaced on the y axis for clarity purposes.

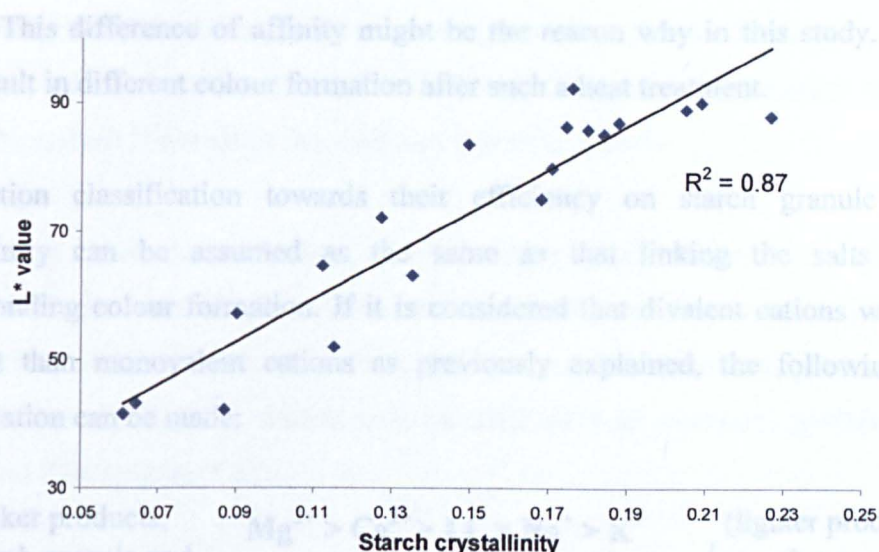
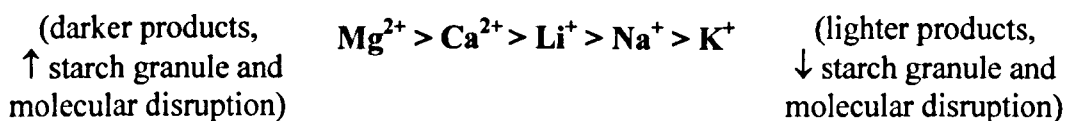


Figure 7.25: L* value of starch samples mixed with different types of salt (0.030 mol of salt / 100 g of dry starch) or mixed with CaCl_2 or MgCl_2 (between 0.015 and 0.025 mol of salt / 100 g of dry starch) and heated at 230 °C for 30 min, versus the sample starch crystallinity (evaluated by calculating the ratio between the area under the peaks and the total area under the curve).

It was observed that the darker the sample, the lower was the crystallinity of the starch granules (Figure 7.25). Based on the previous experiments, this linear correlation between the colour formation and the starch crystallinity of the samples may indicate that the more the crystalline structure was lost, the more the starch macromolecular structures were disrupted and the more the starch molecule might be degraded in a similar way as during pyroconversion. It was already patented that adding CaCl_2 or AlCl_3 to starch granules acted as a second catalyser with acids during starch pyroconversion (Van Beynum and Roels, 1985). Hence, salt addition to starch granules may influence the amount of glucose formed that might then caramelize and generate colour, explaining the different influence of the type of salt on the sample colour formation. Indeed, it was previously reported that the salt/starch interactions changed the thermal properties of the starches (Szymonska et al., 2008; Ciesielski and Tomasik, 2004a; Ciesielski et al., 2003; Lai et al., 2001). Catalytic effects of several salts on amylose (Desai et al., 1972) and potato starch (Ciesielski and Tomasik, 2004b) decomposition were also reported. Moreover, Ciesielski and Tomasik (2003) showed that different starches possessed their own specific affinity to the same metal ion and there was different affinity among metal ions to a given

ligand. This difference of affinity might be the reason why in this study, different salts result in different colour formation after such a heat treatment.

The cation classification towards their efficiency on starch granule loss of crystallinity can be assumed as the same as that linking the salts and the corresponding colour formation. If it is considered that divalent cations were more efficient than monovalent cations as previously explained, the following cation classification can be made:



The classification of the cations' efficiency towards starch breakdown and colour formation of a starch based sample during a heat treatment at 230 °C followed the Hofmeister series (see literature review, section 2.3.2.2). In the present study, the weakly hydrated cations (structure-breakers or chaotropes) had more influence on starch disruption and colour formation ($p < 0.05$) than the strongly hydrated ones (structure-makers or kosmotropes).

The structure breaker cations might have “bonded” somehow with the starch molecules during the mixing stage in the presence of water (the nature of the interactions remains unclear, as discussed in section 2.3.2.2 of the literature review). These “bonds” might be dependent on the nature of the cations and the properties of the starch. The interactions might remain present even after the drying step (to 20 % moisture wwb) and during the toasting step performed on the model systems, changing the thermal stability of the carbohydrates according to the salt's position in the Hofmeister series. Divalent cations might have a stronger impact on starch alteration during a heat treatment, as the divalent cations could be more strongly attracted by the starch granules due to the Donnan potential during the mixing stage. Moreover, half the divalent cation concentration would be required to balance the Donnan potential compared to monovalent cations. The resulting thermal stability might be influenced by the amount of cations present in the granule or the strength of the cation / starch interactions.

The influence of the cations on the water structure seemed to be unlikely in this study to explain starch alteration during the heat treatment, as this alteration began at a stage where most if not all of the moisture was evaporated.

It should be noted that regardless of the position of the cation in the Hofmeister series within the salts studied, it seemed that there was always some interactions between the cations and the starch (to a different extent). Indeed, the presence of salt always led to significantly darker samples after the heat treatment ($p < 0.05$) except for KI and KBr (Figure 7.23).

The anions did not have a significant influence on colour formation, hence it could be assumed they did not influence starch alteration. The absence of impact of the anions on starch alteration might be explained by the fact that anions were excluded from the starch granules due to the Donnan potential (Oosten, 1983). Hence, they did not have any impact on the native starch granule thermal stability.

7.7. Conclusions

When a native or pregelatinised starch sample was heated at 230 °C in an open container, the starch granule and the starch polymer were degraded by the heat treatment. Presence of NaCl enhanced such degradation proportionally to the NaCl concentration (for native and gelatinised starch). This degradation might lead to the formation of smaller molecules such as glucose. The use of high temperatures (230 °C) could then be ideal conditions for caramelisation of the formed glucose, leading to colour formation, which could explain the influence of NaCl on colour formation in cereal based products. This influence of NaCl on starch degradation might be due to an interaction between the sodium ion and the starch molecule. X-ray measurements demonstrated that regardless of the moisture content of a starchy system, 5 % NaCl in the system did not lead to any NaCl crystal formation. This might indicate some binding between the sodium and/or the chloride ions and the starch molecule. Other types of salts had similar impacts on starch alteration as NaCl. Addition of salts to a system enhanced starch granule degradation (loss of

crystallinity) and colour formation, but the extent of this enhancement depended on the position of the cation in the Hofmeister series. Varying the extent of starch degradation might vary the extent of glucose formation, which could then caramelize and generate colour during the heat treatment.

It was observed that reducing the amount of NaCl in cereal based products such as breakfast cereals decreased the colour formation, which is in accordance with the present study. Hence, when the sodium chloride content of cereal based products is reduced by manufacturers, other types of salt might be able to compensate for any potential loss of colour formation.

Another way for the salts to influence colour formation, other than by accelerating starch degradation, would be to change the rate of the Maillard or the caramelisation reactions. The salts studied were NaCl, KCl, MgCl₂ and CaCl₂. They were chosen as all of them were edible, and could therefore be potential NaCl replacers in starch based foods.

8. Influence of several salts on colour formation via caramelisation and Maillard reactions

8.1. Introduction

It was previously observed that the presence of NaCl in starchy foods such as breakfast cereals, or in model systems, led to more colour formation (darker products) when they were heated above 180 °C in a dry environment. To understand how NaCl can have such an impact on colour formation, different model systems were used.

Chapter 7 demonstrated that the presence of NaCl with starch during a toasting treatment might enhance the starch granule and the starch molecule alteration. This could lead to the formation of glucose (due to a starch breakdown), which might then caramelize during this heat treatment, generating colour. Hence, colour formation in breakfast cereals was enhanced by the presence of NaCl.

Colour formation in the food product might also come from the caramelisation of the sugars initially present or formed during the toasting stage and from the Maillard reactions. It might be possible that NaCl has a direct impact on both these reactions. If NaCl has a direct impact on caramelisation and/or Maillard reactions, other salts might have a similar behaviour. The main goal of this project was to find ways to reduce the sodium content in breakfast cereals. Hence, it is essential to broaden the study of NaCl to other types of salts to see whether some other compounds have similar impacts as NaCl on some properties of breakfast cereal products, such as colour. If that was the case, a replacement of NaCl by such salts could be considered to compensate for some quality loss due to a sodium reduction.

8.2. Aims

This chapter focused on the impact of NaCl, KCl, CaCl₂ and MgCl₂ on colour formation via caramelisation or Maillard reactions. These three other salts were chosen as they were edible and they seemed to impact on starch disruption in a similar way (or even more efficiently for CaCl₂ and MgCl₂) to NaCl.

Maillard and caramelisation reactions were studied separately by the use of different model systems. A model composed of only glucose and salts was used to focus on caramelisation reactions. Glucose, amino-acids and salts were mixed and heated to generate Maillard reactions. In this case, caramelisation reactions were considered negligible. Indeed, it was previously reported that in equimolar solutions of asparagine and glucose heated between 120 and 200 °C, caramelisation reactions were negligible (De Vleeschouwer et al., 2008). The influence of moisture content on colour generation via Maillard reactions was also observed.

8.3. Salt's influence on colour formation via caramelisation

8.3.1. System preparation

Solutions of glucose and NaCl (between 0 and 4 % dwb) at 20 % moisture (wwb) were prepared according to the procedure presented in section 3.2.1.3. A blank was made where no salt was added. Ten grams of each of these solutions were heated at 180 °C for 18 min or at 230 °C for 11 min.

Solutions of glucose, salt (NaCl, KCl, CaCl₂ or MgCl₂ between 0.023 and 0.090 mol of salt / mol of glucose dwb) and water (to achieve a moisture content of 20 % wwb) were prepared in a similar way. A blank was made where no salt was added. Ten grams of these solutions were heated at 230 °C for 8 min. The samples were then dissolved with 10 g of distilled water for further analysis.

8.3.2. Results and discussion

Solutions of glucose and NaCl (between 0 and 4 % dwb) were heated at 180 °C for 18 min or at 230 °C for 11 min. Pictures of the samples are presented in Figure 8.1.

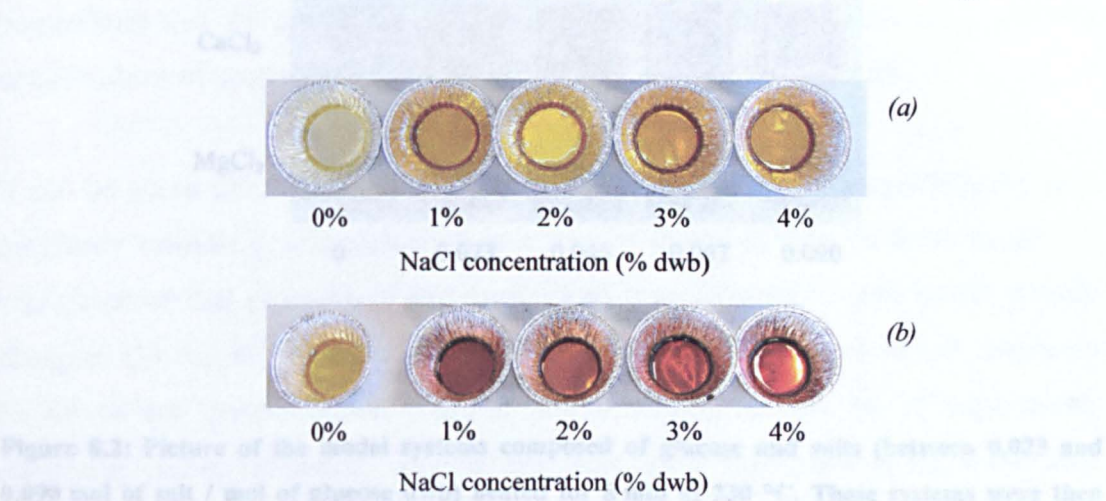


Figure 8.1: Picture of the solutions of glucose and NaCl (between 0 and 4 % dwb) heated at 180 °C for 18 min (a) or at 230 °C for 11 min (b).

According to these model systems, presence of NaCl seemed to enhance colour generation via caramelisation reactions within the heating times and temperatures used.

It was previously reported that some compounds are known catalysts for caramelisation reactions, such as ammonia and ammonium salts, acids, sulfites, hydroxides and basic amino-acids (Fadel and Farouk, 2002; Defaye and Ratsimba, 2000; Sikora and Krakow, 1994; Pons et al., 1991; Sikora and Tomasik, 1989). Even though NaCl was not among the list of catalysts reported to increase the rate of caramelisation reaction, it might not be surprising to observe such an impact of NaCl on colour formation via caramelisation.

Solutions of glucose, a salt (NaCl, KCl, CaCl₂ or MgCl₂) and water (20 % moisture ww) were heated at 230 °C for 8 min. These samples were then dissolved in 10 ml of water after the heat treatment for further analysis. A picture of the resulting samples is presented in Figure 8.2. The colour of the solutions was measured and the data are shown in Table 8.1.

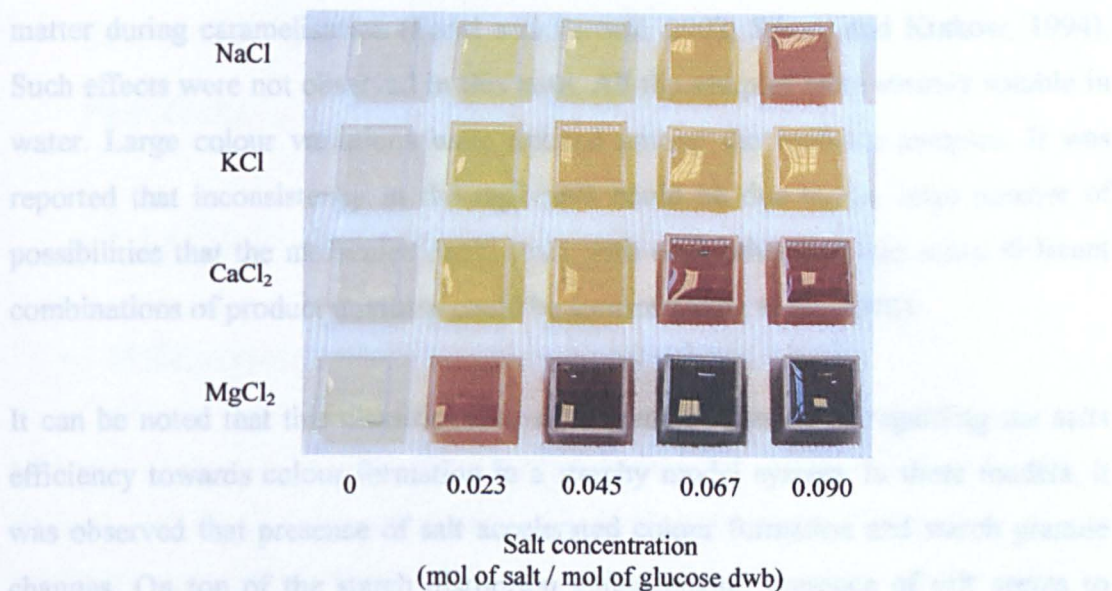


Figure 8.2: Picture of the model systems composed of glucose and salts (between 0.023 and 0.090 mol of salt / mol of glucose dwb) heated for 8 min at 230 °C. These systems were then dissolved in 10 ml of distilled water.

Table 8.1: L* value of samples composed of glucose, water and salt heated at 230 °C for 8 min and dissolved in 10 ml of distilled water. Samples were done in triplicates and colour measurements were also performed in triplicates. The letters next to the L* values indicate significant differences between the samples ($p < 0.05$).

Salt concentration (mol of salt / mol of glucose dwb)	0	0.023	0.045	0.067	0.090
NaCl	99.36 ^a	98.22 ^a	95.78 ^{a, b}	95.63 ^{a, b}	91.07 ^b
KCl	99.36 ^a	98.04 ^a	97.84 ^a	96.41 ^a	83.42 ^b
CaCl ₂	99.36 ^a	94.33 ^{a, b}	91.16 ^{a, b, c}	87.09 ^{b, c}	83.75 ^c
MgCl ₂	99.36 ^a	69.25 ^{a, b}	46.02 ^b	39.73 ^b	38.93 ^b

Presence of a salt in a glucose solution heated for 8 min at 230 °C always led to darker products ($p < 0.05$). Hence, presence of salt accelerated colour formation via caramelisation. However, the salt concentration threshold at which the colour formed became significantly different from the glucose solution heated without salt depended on the type of salt added (Table 8.1). From the least to the most efficient salt regarding colour formation, the following classification can be made: $\text{KCl} < \text{NaCl} < \text{CaCl}_2 < \text{MgCl}_2$. It was also reported that sodium salts led to soluble coloured compounds, while calcium and magnesium salts led to the formation of insoluble

matter during caramelisation (Fadel and Farouk, 2002; Sikora and Krakow, 1994). Such effects were not observed in this case. All the samples were entirely soluble in water. Large colour variations were noticed among the replicate samples. It was reported that inconsistency in the replicates could be due to the large number of possibilities that the molecules could react with each other and that many different combinations of product mixtures could be formed (Jiang et al., 2008).

It can be noted that this classification is the same as that made regarding the salts efficiency towards colour formation in a starchy model system. In these models, it was observed that presence of salt accelerated colour formation and starch granule changes. On top of the starch disruption enhancement, presence of salt seems to accelerate the caramelisation reaction rate according to this set of experiment, enhancing colour formation. Both mechanisms could take place at the same time during the heat treatment of a starch based food containing NaCl.

It might be suggested that one mechanism could also be the consequence of the other. Indeed, caramelisation reactions cause the release of H^+ . Thus, the pH of the system undergoing caramelisation falls with time (Kroh, 1994). Addition of acids to starches before a heat treatment is a well known way to produce dextrins and glucose as it accelerates starch degradation (pyroconversion) (Tomasik et al., 1989; Van Beynum and Roels, 1985). Therefore, another way for salts to enhance starch degradation during a heat treatment would be at first to accelerate the caramelisation reactions, hence the production of acids. This initial caramelisation would come from the glucose formation due to starch degradation caused by the heat treatment. These H^+ released would then accelerate starch degradation and the production of glucose which would then caramelize, producing more acids, etc. Starch granule disruption being a consequence of enhanced caramelisation reaction with NaCl could as well explain the same observed classification of the salts towards colour formation efficiency.

Colour formation in breakfast cereal products could also be due to Maillard reactions. The influence of several salts on colour formation via this type of reaction is presented in the following section.

8.4. Salt’s influence on colour formation via Maillard reactions

8.4.1. System preparation

- Model systems composed of glucose, amino-acids and salts

Mixtures of glucose, amino-acids (leucine, valine, asparagine, glutamic acid and alanine), and NaCl (between 0 and 5 % dwb) were prepared according to the procedure presented in section 3.2.1.4. These mixtures were made in order to have a homogeneous compound repartition within the system and a final moisture content between 5 and 40 % (wwb). The number of samples, their NaCl concentrations and moisture contents followed the experimental design presented in Table 8.2. This experimental design was used to observe colour formation versus NaCl concentration and moisture content for a heat treatment of 3 min at 180 °C or 5.30 min at 180 °C. Hence, two sets of data were obtained. The samples were dissolved in 50 ml of distilled water after the heat treatment for further analysis.

Mixtures of glucose, amino-acids (leucine, valine, asparagine, glutamic acid and alanine), and salts (NaCl, KCl, CaCl₂ or MgCl₂ between 0 and 6 % dwb) with a moisture content of 20 % (wwb) were prepared in a similar way as the previous samples. The samples were heated for 1.5 min at 230 °C. They were then dissolved in 50 ml of distilled water for further analysis.

Table 8.2: Experimental design used to study the influence of NaCl concentration and moisture content on colour formation via Maillard reactions for heat treatments of 3 min at 180 °C or 5.30 min at 180 °C.

Run Number	NaCl concentration (% dwb)	Moisture content (% wwb)
1	0.00	40.00
2	3.75	31.25
3	5.00	5.00
4	1.67	5.00
5	3.33	40.00

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6	2.50	22.50
7	5.00	40.00
8	2.50	22.50
9	1.67	40.00
10	0.00	22.50
11	0.00	40.00
12	5.00	40.00
13	5.00	16.67
14	0.00	5.00
15	5.00	5.00
16	5.00	28.33
17	3.33	5.00
18	3.75	13.75
19	0.00	5.00
20	0.00	22.50
21	2.50	22.50
22	1.25	31.25
23	1.25	13.75

- Model systems composed of starch, amino-acids and NaCl

Model systems containing starch, amino-acids and NaCl were made according to the procedure detailed in section 3.2.1.1. The model system compositions are shown in Table 8.3.

Table 8.3: Model system composition before the drying step.

NaCl concentration (% dwb)	0.00	1.35	2.72	4.09	5.44
Native maize starch (g wet weight)	50.117	49.492	73.300	48.242	47.617
Leucine (g)	0.147	0.147	0.221	0.147	0.147
Valine (g)	0.054	0.054	0.082	0.054	0.054
Alanine (g)	0.032	0.032	0.048	0.032	0.032
Glutamic acid (g)	0.516	0.516	0.774	0.516	0.516
Distilled water (g)	49.134	49.134	73.696	49.134	49.134
NaCl (g)	0.000	0.625	1.879	1.875	2.500
Total (g)	100.000	100.000	150.000	100.000	100.000

After drying, the moisture content of the mixture was adjusted to 20 % (wwb). Aliquots of 12 g were heated for different periods of time (0 to 25 min) at 230 °C, according to the experimental design presented in Table 5.2. This set of data was then compared to the system containing starch, amino-acids, glucose and NaCl, which was described in Chapter 5 (Table 5.1) and was also heated according to the design presented in Table 5.2.

8.4.2. Results and discussion

8.4.2.1. NaCl influence on colour formation via Maillard reactions

Mixtures of amino-acids, glucose (glucose/amino-acid molar ratio of 1/1) and NaCl (between 0 and 5 % dwb) at moisture contents between 5 and 40 % (wwb) were heated for 3 min or 5.30 min at 180 °C. Results are presented in Figure 8.3 and Figure 8.4.

In both experimental designs, NaCl concentration and initial moisture content had a significant influence on the L^* values of the model systems. Increasing NaCl concentration inhibited colour formation via Maillard reactions. This trend was the same regardless of the moisture content used, but colour differences were more noticeable at higher moisture contents for the heating times and temperatures used. Indeed, at low moisture content, the systems might have taken less time to reach the oven temperature as less water evaporated. Hence, this might explain why the systems with a lower moisture content became darker compared to the ones with a higher moisture content for similar heat treatments. However, the influence of NaCl was the same regardless of the moisture content.

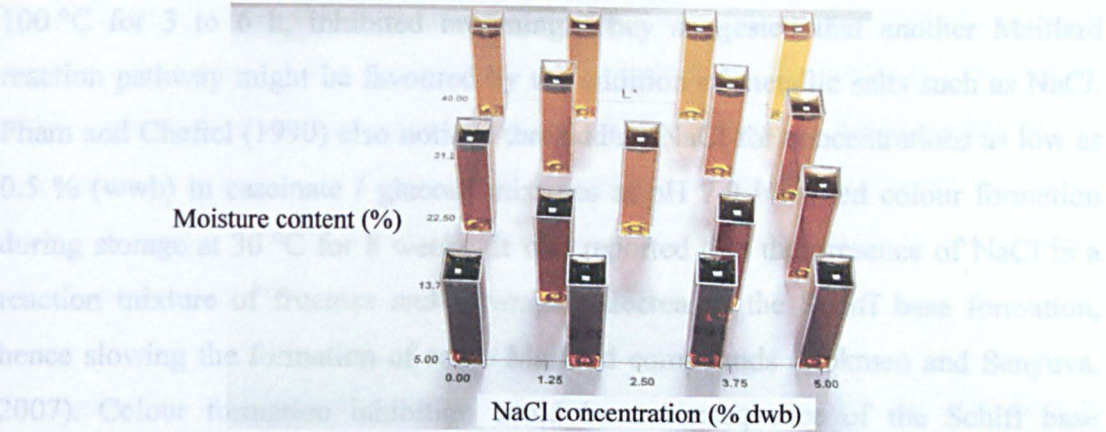


Figure 8.3: Mixtures of amino-acids, glucose and NaCl (between 0 and 5 % dwb) at moisture contents between 5 and 40 % (wwb) heated for 3 min at 180 °C. The following equation was calculated: $L^* = 27.93 + 1.49 * \text{NaCl concentration} + 1.89 * \text{Moisture content} - 0.018 * \text{Moisture content}^2$, $r^2 = 0.93$.

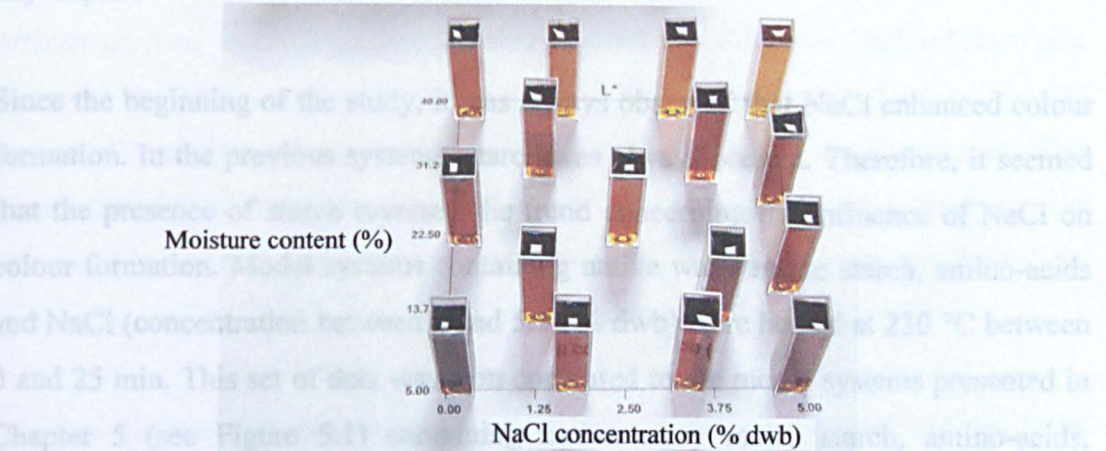


Figure 8.4: Mixtures of amino-acids, glucose and NaCl (between 0 and 5 % dwb) at moisture contents between 5 and 40 % (wwb) heated for 5.30 min at 180 °C. The following equation was calculated: $L^* = 21.62 + 1.04 * \text{NaCl concentration} + 1.45 * \text{Moisture content} - 0.015 * \text{Moisture content}^2$, $r^2 = 0.86$.

These systems had a pH of 3.5, which was lower than the pH of breakfast cereals. As pH is an important factor affecting Maillard reactions, one could object that these model systems did not reflect breakfast cereal products. However, such influence of NaCl on colour formation via Maillard reactions was already reported by Kwak and Lim (2004) in glucose and amino-acid solutions at pH 6.5, pH similar or even above the pH of breakfast cereals. In the Kwak and Lim (2004) paper, it was concluded that adding NaCl (1 to 10 %) to sugar and amino-acid solutions, that were heated at

100 °C for 3 to 6 h, inhibited browning. They suggested that another Maillard reaction pathway might be favoured by the addition of metallic salts such as NaCl. Pham and Cheftel (1990) also noticed that adding NaCl for concentrations as low as 0.5 % (wwb) in caseinate / glucose mixtures at pH 7.0 inhibited colour formation during storage at 30 °C for 8 weeks. It was reported that the presence of NaCl in a reaction mixture of fructose and asparagine decreased the Schiff base formation, hence slowing the formation of some Maillard compounds (Gokmen and Senyuva, 2007). Colour formation inhibition could be a consequence of the Schiff base formation decrease. However, the influence of salts on colour formation via Maillard reactions might change depending on the type of amino-acids or proteins used. Indeed, Kato et al. (1981) observed that Fe^{3+} and Cu^{2+} accelerated colour formation in freeze-dried ovalbumin-glucose systems stored at 50 °C, while Na^+ did not have any impact.

Since the beginning of the study, it was always observed that NaCl enhanced colour formation. In the previous systems, starch was always present. Therefore, it seemed that the presence of starch reversed the trend concerning the influence of NaCl on colour formation. Model systems containing native waxy maize starch, amino-acids and NaCl (concentration between 0 and 5.44 % dwb) were heated at 230 °C between 0 and 25 min. This set of data was then compared to the model systems presented in Chapter 5 (see Figure 5.1) containing native waxy maize starch, amino-acids, glucose and NaCl (same concentrations) and heated under the same conditions. Pictures comparing both sets of model systems are presented in Figure 8.5.

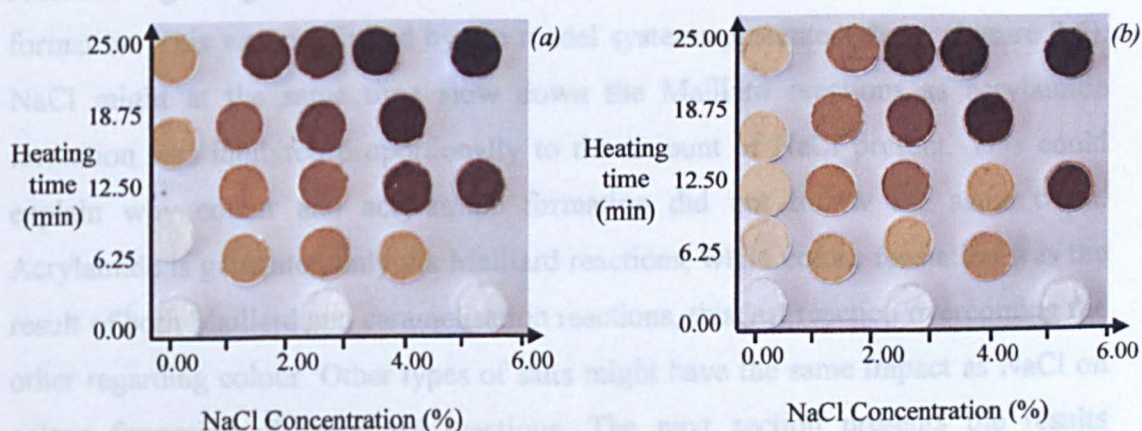


Figure 8.5: Picture of model systems containing starch, amino-acids, glucose and NaCl (a) or starch, amino-acids and NaCl (b) heated at 230 °C versus heating time and NaCl concentration.

The difference between the two sets of model systems presented in Figure 8.5 is the absence of glucose in the model systems presented on the right. In the system without glucose, caramelisation reactions should be minimal at the beginning of the heat treatment as no sugars were present. Indeed, colour formation was more rapid in the set of model systems containing glucose. This was predictable as the glucose initially present could caramelise and generate colour.

Caramelisation reactions could happen in the systems without glucose only after a certain period of time, due to a possible monossacharide formation via starch breakdown. However, when glucose might be generated due to starch disruption, the presence of a large amount of amino-acids compared to the amount of glucose could favour the Maillard reactions compared to caramelisation reactions. If that was the case, colour formation should be lower at high NaCl concentrations in the system without glucose, as NaCl seemed to inhibit colour formation via Maillard reactions. However, for high heating times and high NaCl concentrations, the same colour was generated regardless of the amount of glucose present in the model systems. This demonstrated that even though both Maillard and caramelisation can happen at the same time in a system, caramelisation was favoured, as assessed by colour formation, even in the presence of a large amount of amino-acids.

One can assume that in breakfast cereals, caramelisation and Maillard reactions happen at the same time as starch, reducing sugars and proteins are all present in the system. Starch degradation and/or caramelisation seemed to dominate over Maillard reactions regarding colour formation in breakfast cereals, as NaCl enhanced colour formation. This was confirmed by the model systems presented above (Figure 8.5). NaCl might at the same time slow down the Maillard reactions as acrylamide formation was inhibited proportionally to the amount of NaCl present. This could explain why colour and acrylamide formation did not follow the same trend. Acrylamide is generated only via Maillard reactions, while colour formation was the result of both Maillard and caramelisation reactions, this last reaction overcoming the other regarding colour. Other types of salts might have the same impact as NaCl on colour formation via Maillard reactions. The next section presents the results concerning the influence of KCl, CaCl₂ and MgCl₂ on colour formation via Maillard reactions.

8.4.2.2. *NaCl, KCl, CaCl₂ and MgCl₂'s influence on colour formation via Maillard reactions*

Model systems containing amino-acids, glucose (glucose/amino-acids molar ratio of 1/1) and NaCl, KCl, CaCl₂ or MgCl₂ (between 0 and 6 % dwb) with a moisture content of 20 % (wwb) were heated for 1.5 min at 230 °C. The amounts of glucose and amino-acids were the same in each sample. After the heat treatment, the samples were dissolved in 50 g of distilled water. A picture of these models is presented in Figure 8.6. Colour measurements were then performed. Results are presented in Figure 8.7.

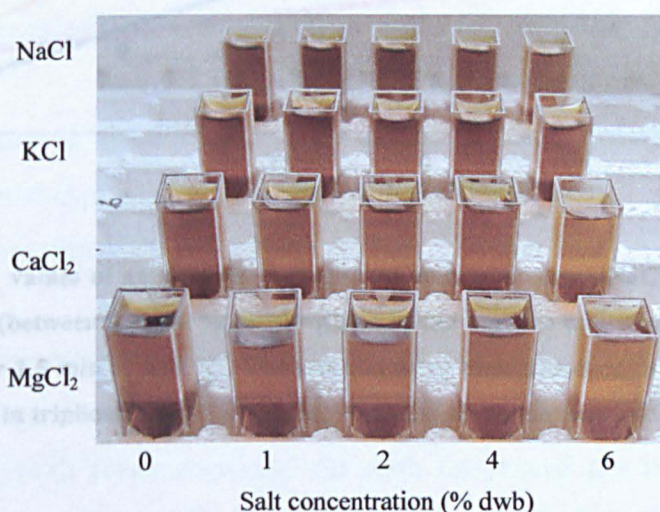


Figure 8.6: Mixtures of amino-acids, glucose and NaCl, KCl, CaCl₂ or MgCl₂ (between 0 and 6 % dwb) with a moisture content of 20 % (wwb) heated for 1.5 min at 230 °C. The samples were then dissolved in 50 g of distilled water.

Statistical analyses (ANOVA) on the L* values of the model systems demonstrated that presence of NaCl, CaCl₂ and MgCl₂ had a significant influence on colour formation for the heating times and temperatures used ($p < 0.05$). By contrast, the presence of KCl did not influence significantly the L* values of the systems ($p > 0.05$), even though the average L* value increased when the KCl concentration increased. Higher KCl concentrations might be needed for its influence on colour formation to become significant. Increasing the NaCl concentration decreased colour formation when the systems were heated for 1.5 min at 230 °C. This influence became significant for a NaCl concentration of 6 % (dwb). This confirmed the previous results and demonstrated that the heating temperatures used (between 180

and 230 °C) did not change the influence of NaCl on colour formation via Maillard reactions.

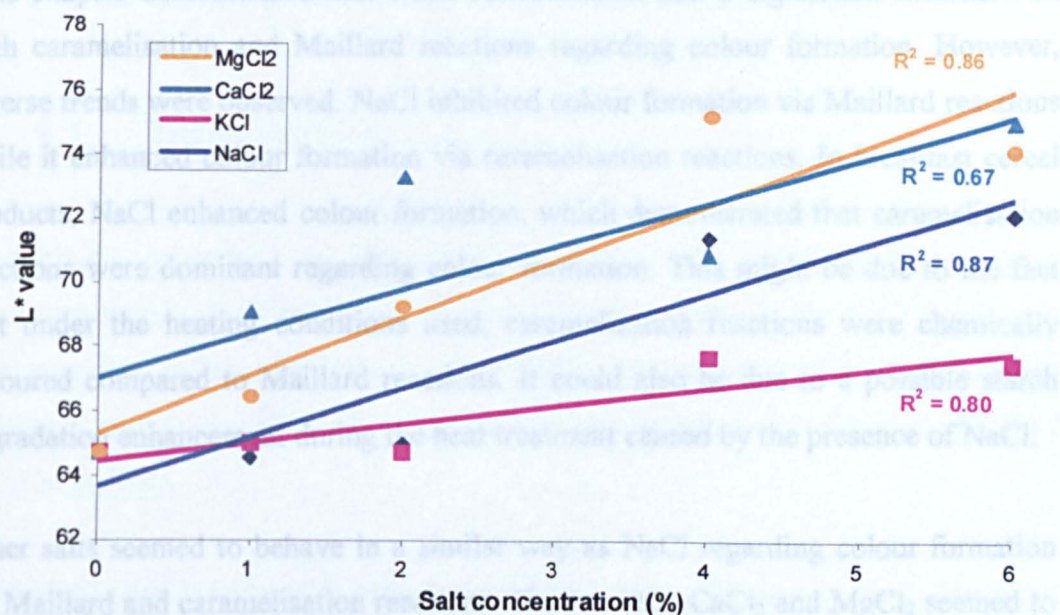


Figure 8.7: L* values of amino-acids and glucose mixtures versus NaCl, KCl, CaCl₂ or MgCl₂ concentration (between 0 and 6 % dwb) with an initial mixture moisture content of 20 % (wwb) and heated for 1.5 min at 230 °C. The samples were then dissolved in 50 g of distilled water. Samples made in triplicates and the average standard deviation was 2.65.

Increasing the concentration of CaCl₂ and MgCl₂ inhibited colour formation via Maillard reactions. This influence became significant for a concentration of 1 % (dwb) for both these salts. Lindsay and Jang (2005) suggested that salts like CaCl₂ or FeCl₃ could be associated with the amino-acids via ionic interactions, minimizing the early-stage of Maillard reactions. It was also found that addition of cations such as Ca²⁺ and Mg²⁺ would change the reaction path from the Maillard reactions toward dehydration of glucose (Gokmen and Senyuva, 2007). These salts' influence on the amino-acids or on the Maillard reactions pathways could have some repercussions on colour formation, such as its inhibition.

This set of experiments demonstrated that NaCl, as well as some other salts, seemed to slow down Maillard reactions in model systems. This corroborates the previous findings concerning acrylamide formation in breakfast cereals, which was decreased by the presence of NaCl.

8.5. Conclusions

This chapter demonstrated that NaCl concentration had a significant influence on both caramelisation and Maillard reactions regarding colour formation. However, reverse trends were observed. NaCl inhibited colour formation via Maillard reactions while it enhanced colour formation via caramelisation reactions. In breakfast cereal products, NaCl enhanced colour formation, which demonstrated that caramelisation reactions were dominant regarding colour formation. This might be due to the fact that under the heating conditions used, caramelisation reactions were chemically favoured compared to Maillard reactions. It could also be due to a possible starch degradation enhancement during the heat treatment caused by the presence of NaCl.

Other salts seemed to behave in a similar way as NaCl regarding colour formation via Maillard and caramelisation reactions. The two salts CaCl_2 and MgCl_2 seemed to have even more influence on colour formation than NaCl.

Colour was the marker chosen to follow Maillard and caramelisation reactions for practical reasons. However, it should be kept in mind that hundreds of molecules are generated by both these reactions. As salts influenced the melanoidin formation (colour formation), it might be possible that they could also influence the generation of other types of molecules, such as molecules responsible for aroma and flavour.

Hence, presence of NaCl in breakfast cereals might not be responsible only for the salty taste of the products, but also impact the generation of other flavour molecules. If that was the case, decreasing or removing the NaCl from the recipes might alter the overall flavour of the products. As CaCl_2 and MgCl_2 seemed to impact caramelisation and Maillard reactions in a similar way as NaCl, adding these salts to breakfast cereals might compensate for a potential flavour loss due to a decrease in NaCl concentration. The next chapter exposes the characteristics of breakfast cereal products containing CaCl_2 and MgCl_2 .

9. NaCl replacement in breakfast cereals

9.1. Introduction

In western countries, as the sodium daily intake exceeds the medical recommendations, food industries are encouraged to decrease the NaCl content of their products. In breakfast cereals, the sodium content target set by the FSA was 300 mg of sodium per 100 g of product, which corresponds to a NaCl level of 0.8 g / 100 g of product.

If the NaCl level is reduced in breakfast cereal products without any other changes in the process or the recipe, the taste of the product becomes less salty and the overall flavour is described as bland. Indeed, in bakery products, NaCl is added for its salty taste but also to “round out” the overall flavour sensation (Reddy and Marth, 1991). Hence, the formulation and/or the process of breakfast cereals have to be adjusted when the NaCl level is reduced in order to have products that are acceptable to the consumer.

It was previously observed that NaCl influenced the colour formation generated via Maillard and caramelisation reactions. This suggested that NaCl modified the rates and/or the pathways taken by these reactions. Therefore, in breakfast cereal products, Maillard and caramelisation reactions might be altered when the NaCl level is reduced. This might explain why reducing the NaCl concentration does not affect only the salty taste of the product but also the overall flavour, as Maillard and caramelisation reactions are responsible for the formation of hundreds of molecules responsible for the aroma and flavour of a product. Other salts, such as CaCl_2 and MgCl_2 , seemed to influence Maillard and caramelisation reactions regarding colour formation in a similar or even more efficient way as NaCl. One could hypothesise that these salts, when added to breakfast cereal products, might restore some of the potential flavour loss caused by a reduction of the NaCl content.

9.2. Aims

The main goal of this study was to create low NaCl breakfast cereal products with an improved taste. The salts CaCl_2 and MgCl_2 were used to see whether they could compensate for the flavour loss due to NaCl reduction as they seemed to influence Maillard and/or caramelisation reactions. This work was performed only on the mixture of wheat and rice cereal products. The product recipes were created step by step as explained:

- (1) Observe the impact of the total replacement of NaCl by CaCl_2 and MgCl_2 (for different concentrations) on breakfast cereals during the process and on the final product characteristics (colour and flavour)
- (2) Acrylamide formation was followed to see whether the acrylamide content was affected by such recipe changes

9.3. System preparation

Wheat and rice mixture breakfast cereal products were made using the pilot plant facilities of CPW. The products were steam-cooked for 65 min at a pressure of 1.2 bars. The moisture content of the product after the cooking step was around 30 % (wwb). The products were then dried and shaped into pellets. The pellets were flattened into flakes before entering the toasting step. The moisture content of the wet flakes was then around 20 % (wwb).

The wet flakes were toasted at 230 °C in the first half of the tunnel and at 190 °C in the second half. This process was previously explained in more details in section 3.2.1.5.

- Influence of MgCl_2 and CaCl_2 in breakfast cereal products

Several salt levels were tried in breakfast cereal products, as shown in Table 9.1. Recipes with a high NaCl level (1.17 % dwb), an intermediate NaCl level (0.75 %) as well as a recipe without salt were made. The amounts of salt added or removed from the recipes were considered negligible compared to the weight of the other ingredients. Hence, no changes were made concerning the quantity of the other ingredients.

Table 9.1: Salt levels used in breakfast cereal recipes.

	NaCl concentration (% dwb)	CaCl_2 concentration (% dwb)	MgCl_2 concentration (% dwb)
High NaCl recipe	1.17	0.00	0.00
Intermediate NaCl recipe	0.75	0.00	0.00
No NaCl recipe	0.00	0.00	0.00
High CaCl_2 recipe	0.00	0.43	0.00
Low CaCl_2 recipe	0.00	0.21	0.00
High MgCl_2 recipe	0.00	0.00	0.43
Low MgCl_2 recipe	0.00	0.00	0.21

A part of each product was processed entirely in the pilot plant, as previously explained. These final products were those presented to the panel for sensory evaluation. The other part of the product was processed in the pilot plant until the stage where wet flakes were produced. These wet flakes were then toasted, using the small scale toasting device presented in section 3.2.1.5, for between 1 and 10 min at temperatures between 180 and 230 °C.

- Breakfast cereal recipe improvements

Based on the results obtained with the trials presented above, the salt levels were adjusted to try to improve the processability of the products (Table 9.2). These products were processed in the pilot plant until the stage where wet flakes were produced. These wet flakes were then toasted for 1 min at 230 °C in the small scale toasting device. This toasting treatment was supposed to mimic the treatment applied

to the products in the toasting tunnel. The acrylamide content of the resulting samples was then measured.

Table 9.2: Salt levels used in breakfast cereal recipes.

	NaCl concentration (% dwb)	MgCl ₂ concentration (% dwb)
Low NaCl recipe	0.75	0
Low NaCl recipe + MgCl ₂	0.75	0.27

- Sensory evaluation of the products

The sensory evaluation of the breakfast cereal products was performed with the sensory department of CPW. The assessors chosen for this study were previously trained by the sensory department of CPW over a six month period on sensory evaluation and specific sensory characteristics of breakfast cereals and their ingredients. During the training, references were provided to acquaint the panellists with the main characteristics of breakfast cereal products (such as those evaluated in this study). These references included wheat, corn, rice and oats that were raw, cooked or toasted. The panel was also trained to quantify flavour and aroma characteristics.

In this study, six trained assessors computed the flavour profile of breakfast cereal products. Seven attributes were evaluated: overall flavour, toasted flavour, salty flavour, sweet flavour, bitter flavour, drying flavour and off flavour. Aromas (overall aroma and toasted aroma) were two attributes that were also tested during some sensory evaluations. Assessors reported their notations on an arbitrary scale, which were converted to a value from 0 to 100. Prior to testing, assessors were presented with reference samples to show the range of samples in the set. They were told which sample was the reference, but were given no details about the other samples, or about the objective of the test.

Panellists from the expert panel then assessed the products twice. Samples were served in a polystyrene bowl, and tasted dry. Panellists were presented with a labelled reference, which they were asked to give a score of 50 (middle of the scale).

The labelled reference was kept by the assessors throughout the session to be able to compare the other samples to this product. All the samples were then tested sequentially with a randomised presentation. Panellists were asked to directly compare the tested samples to the labelled reference. They were also asked to note down any specific comments they had. To help eliminate problems with flavour build-up or carry-over, panellists were forced to take a 90 sec break between samples, and cleansed their palates with water. To help eliminate fatigue, no more than four samples were seen at any one time, and longer breaks of 15 min were taken in between blocks of four samples.

An ANOVA test was run for each attribute to evaluate whether the differences between the products (including the reference) were significant concerning this specific attribute. A blind reference (reference product which was not labelled as a reference but as a product to evaluate) was also included among the products to test. This blind reference was used to check whether the assessors could score it as not significantly different to the reference, which confirmed the reproducibility of the assessors. After the ANOVA test was performed for each of the attributes tested, the products were compared by a Fishers LSD test (confidence level of 95 %).

9.4. Results and discussion

9.4.1. Influence of CaCl_2 and MgCl_2 on colour and taste of breakfast cereals

9.4.1.1. *Colour formation in breakfast cereals containing CaCl_2 and MgCl_2*

Wet flakes without salt, with 0.75 or 1.17 % of NaCl (dwb) or containing 0.43 % (dwb) of CaCl_2 or MgCl_2 were toasted between 1 and 10 min at temperatures between 180 and 230 °C using the small scale toasting device. The colour of these five toasted products was then measured and compared. Figure 9.1 represents the L^* values of the flakes toasted at 180, 205 and 230 °C for different lengths of time.

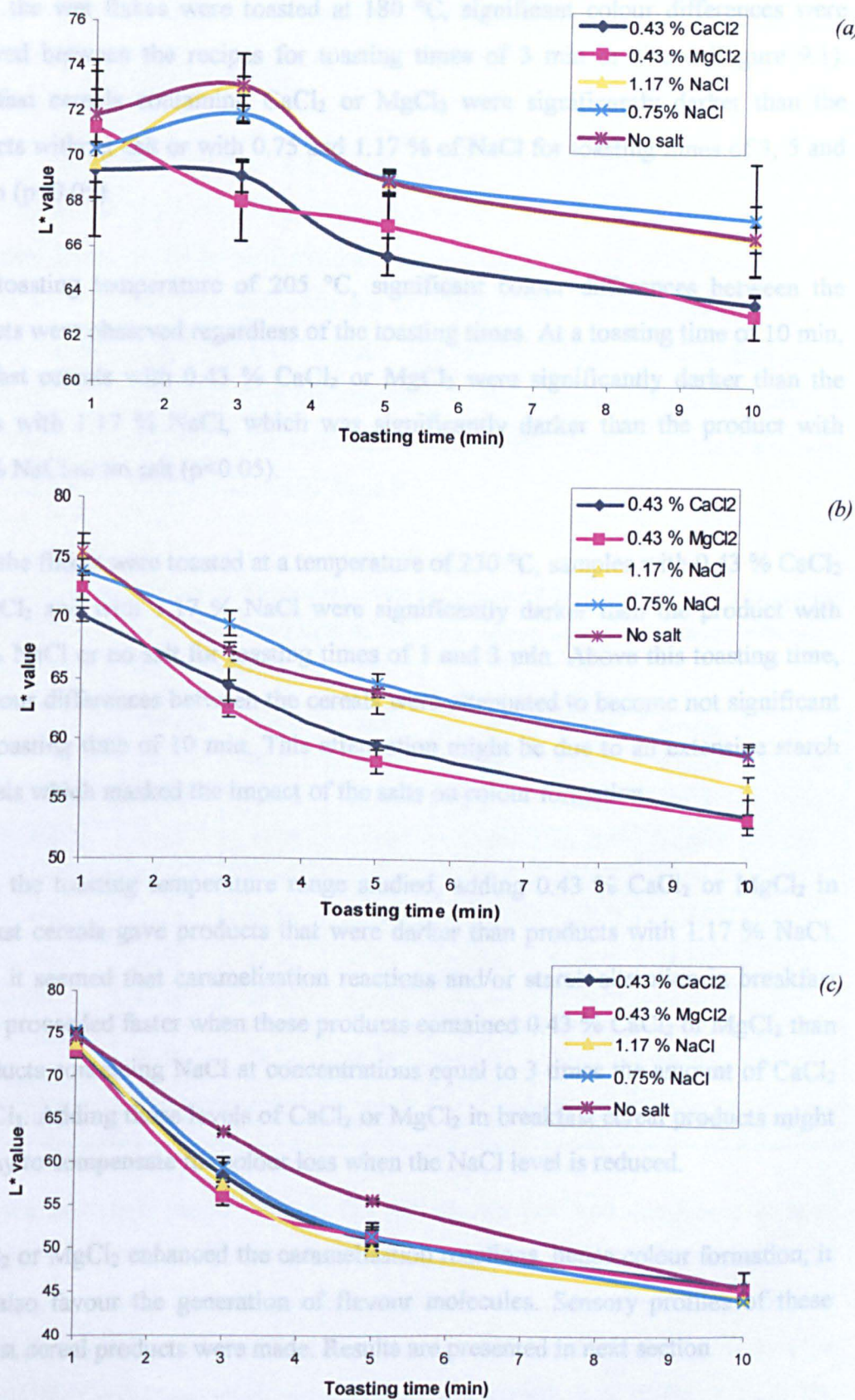


Figure 9.1: L* values of breakfast cereal products toasted between 1 and 10 min at 180 °C (a), 205 °C (b) and 230 °C (c) in the small scale toasting device (samples made in triplicate).

When the wet flakes were toasted at 180 °C, significant colour differences were observed between the recipes for toasting times of 3 min or above (Figure 9.1). Breakfast cereals containing CaCl_2 or MgCl_2 were significantly darker than the products without salt or with 0.75 and 1.17 % of NaCl for toasting times of 3, 5 and 10 min ($p < 0.05$).

At a toasting temperature of 205 °C, significant colour differences between the products were observed regardless of the toasting times. At a toasting time of 10 min, breakfast cereals with 0.43 % CaCl_2 or MgCl_2 were significantly darker than the cereals with 1.17 % NaCl, which was significantly darker than the product with 0.75 % NaCl or no salt ($p < 0.05$).

When the flakes were toasted at a temperature of 230 °C, samples with 0.43 % CaCl_2 or MgCl_2 and with 1.17 % NaCl were significantly darker than the product with 0.75 % NaCl or no salt for toasting times of 1 and 3 min. Above this toasting time, the colour differences between the cereals were attenuated to become not significant for a toasting time of 10 min. This attenuation might be due to an extensive starch pyrolysis which masked the impact of the salts on colour formation.

Within the toasting temperature range studied, adding 0.43 % CaCl_2 or MgCl_2 in breakfast cereals gave products that were darker than products with 1.17 % NaCl. Hence, it seemed that caramelisation reactions and/or starch alteration in breakfast cereals proceeded faster when these products contained 0.43 % CaCl_2 or MgCl_2 than in products containing NaCl at concentrations equal to 3 times the amount of CaCl_2 or MgCl_2 . Adding those levels of CaCl_2 or MgCl_2 in breakfast cereal products might be a way to compensate for colour loss when the NaCl level is reduced.

If CaCl_2 or MgCl_2 enhanced the caramelisation reactions, hence colour formation, it might also favour the generation of flavour molecules. Sensory profiles of these breakfast cereal products were made. Results are presented in next section.

9.4.1.2. Sensory profiles of breakfast cereals containing CaCl_2 and MgCl_2

Products with 0.43 % and 0.21 % CaCl_2 or MgCl_2 , 1.17 % NaCl and no salt, entirely processed in the pilot plant, were evaluated by a trained panel for several flavour attributes. These attributes were overall flavour, toasted flavour, salty flavour, sweet flavour, bitter flavour, drying flavour and off flavour. The reference product was the breakfast cereals containing 1.17 % NaCl. The blind reference was also the product containing 1.17 % NaCl; it was not annotated as the reference, but as a product to score. Statistical analyses of the differences between the products for each attributes were made. Results are presented in Table 9.3.

Table 9.3: Mean scores given to 7 breakfast cereal samples for 7 flavour attributes. The samples were tasted in duplicate. The letters next to the mean scores indicate significant differences between the samples for each attribute ($p < 0.05$).

	Overall flavour	Toasted flavour	Salty flavour	Sweet flavour	Bitter flavour	Drying flavour	Off flavour
1.17 % NaCl	50.00 ^a	50.00	50.00 ^a	50.00	50.00 ^c	50.00 ^b	50.00
0.43 % CaCl_2	45.20 ^{bc}	49.00	37.40 ^b	41.80	56.10 ^{ab}	56.60 ^a	52.60
0.21 % CaCl_2	47.10 ^{ab}	52.50	39.60 ^b	48.30	56.50 ^a	58.20 ^a	52.20
0.43 % MgCl_2	44.50 ^{bc}	45.80	39.30 ^b	48.60	56.80 ^a	58.40 ^a	51.20
0.21 % MgCl_2	46.00 ^{bc}	47.60	39.60 ^b	46.00	53.80 ^{abc}	54.40 ^{ab}	50.80
No salt	42.70 ^c	44.60	36.00 ^b	45.10	52.60 ^{abc}	57.70 ^a	50.50
Blind standard (1.17 % NaCl)	49.80 ^a	52.70	46.50 ^a	49.40	52.10 ^{bc}	53.20 ^{ab}	50.40
Probability	0.004	0.056	>0.001	0.288	0.025	0.028	0.266

The breakfast cereal product which did not contain salt was considered to have significantly less overall flavour than the product with 1.17 % NaCl. Adding CaCl_2 or MgCl_2 increased the overall flavour of the products compared to the one without salt (significant differences obtained for the samples with 0.21 % CaCl_2). The recipe with 0.21 % CaCl_2 was even considered as not significantly different from the reference sample concerning this attribute. Hence, this seemed to confirm that adding these salts might compensate for some flavour loss due to a removal of NaCl from the recipes.

Although not significant, the impact of CaCl_2 and MgCl_2 on the toasted flavour should be noted (column in yellow). Indeed, adding these salts seemed to increase the toasted flavour, especially when CaCl_2 was added compared to the same product without salt. Therefore, CaCl_2 and MgCl_2 might change the rate and/or the pathways taken by the Maillard or the caramelisation reaction, generating flavour molecules which might not be present in the absence of salt. It could also be envisaged that these salts helped the release of flavour molecules and enhanced their perception (salting-out effect).

However, adding CaCl_2 or MgCl_2 to the recipes increased significantly the drying and the bitter flavours of the products compared to the cereals with 1.17 % NaCl, regardless of the concentration used within the range studied. This might be due to the specific taste of the salts. The recipe without salt was also considered to give more drying and bitter flavours than the one with 1.17 % NaCl. This might suggest that NaCl could inhibit these specific flavours.

For the salty flavour, the reference and the blind standard were evaluated as significantly more salty than all the other breakfast cereal recipes. Removing the NaCl from the products significantly decreased the saltiness of these samples. Adding other types of salts such as CaCl_2 or MgCl_2 did not increase the saltiness of the products. These salts did not have any salty taste.

No significant differences between the samples were observed for the attributes sweet and off flavours (columns in grey in Table 9.3). Hence, decreasing the NaCl level or adding CaCl_2 or MgCl_2 to the breakfast cereals within the range studied did not affect the taste of the products regarding these specific attributes.

It should be noted that the blind standard was always noted as similar ($p > 0.05$) to the reference product, which validated the notations given by the assessors.

Replacing NaCl by CaCl_2 or MgCl_2 improved some flavour characteristics of the products but did not compensate entirely the NaCl removal. It was already reported that such a replacement in bread products gave poor taste (Salovaara, 1982).

9.4.1.3. Processability of breakfast cereals containing CaCl_2 and MgCl_2

Products containing 0.43 % of CaCl_2 or MgCl_2 were very sticky; hence this led to processing issues, especially during the lump breaking stage. Mixing the starch granules originating from the cereal grains with the solution of salt (either CaCl_2 or MgCl_2) might have changed the behaviour of these during the cooking step. Indeed, it was previously demonstrated by Lai et al. (2001) that soaking starch granules in these salt solutions led to some partially deformed granules with some leached materials. More starch molecules might have leached out in the presence of 0.43 % of CaCl_2 or MgCl_2 compared to the presence of 1.17 % of NaCl, resulting in a more sticky mass of breakfast cereal dough. Moreover, the increased dough stickiness with CaCl_2 or MgCl_2 might originate from the influence of these salts on starch gelatinisation (section 2.3.3.2). In the lyotropic series, magnesium and calcium cations are distinctly separated from the sodium cation but they are close to each other at the end of the series. Hence, it might not be surprising that CaCl_2 or MgCl_2 behaved similarly concerning their impact on starch and on dough stickiness, and so differently compared to NaCl. Products with a lower amount of salt (0.21 % CaCl_2 or MgCl_2) did not demonstrate any processing issues.

The sensory tests reported above were performed only after the trials presented in the following section, using blends of salts, had been carried out. It was decided, based on internal sensory tasting sessions that future work would be carried only on MgCl_2 . However, according to the sensory profiles, some work on CaCl_2 might be of interest as this salt seemed to help increase the overall flavour of breakfast cereal products.

9.4.2. Processability and acrylamide content of improved breakfast cereal recipes

In order to improve the processability of breakfast cereal products containing MgCl_2 , a blend of salts was tried. It was decided that a level of 0.75 % NaCl would be used. Indeed, it was previously demonstrated that adding CaCl_2 or MgCl_2 did not increase

the salty taste of the products (these salts do not have a salty flavour). Therefore, the presence of NaCl seemed indispensable to have a salty flavour in breakfast cereals. The level of NaCl used was within the limits set by the FSA.

MgCl₂ was used at a level of 0.27 % in the following recipe. This level was decided upon the previous trials, as such a low concentration was enough to improve the overall flavour of the product, but did not lead to any processing issues. A level of 0.21 % was used in the previous trials. This amount was increased a little to try to keep a product which was not too sticky and would be easily processable, but might give the products higher overall flavour than the products with only 0.21 % MgCl₂. Hence, the following products were made:

- Recipe with 0.75 % NaCl
- Recipe with 0.75 % NaCl + 0.27 % MgCl₂

The breakfast cereal recipe containing 0.75 % NaCl did not present any processing issues, even though the dough was considered as “a bit loose”. When the dough is not sticky enough, this could lead to a bad grain adhesion during pellet formation and lots of fines might be generated during the toasting stage. Adding 0.27 % MgCl₂ to the product gave a very sticky product. It was interesting to observe that a small increase (between 0.21 to 0.27 %) gave products with a different texture. This high stickiness led to some processing issues during the lump breaking stage. An intermediate MgCl₂ concentration might be envisaged for future trials.

Modifying the recipe of breakfast cereal products might affect the level of by-products generated during the process, such as acrylamide. This compound, formed during Maillard reactions, might be affected by the level of salt added in the recipe. Indeed, it was previously observed that these compounds seemed to modify the rate or the pathways of the Maillard reactions. In order to observe acrylamide formation in the products previously tested, the wet flakes containing 0.75 % NaCl and 0.75 % NaCl + 0.27 % MgCl₂ were toasted for 1 min at 230 °C in the small scale toasting device. This heat treatment was considered similar as that applied to the products in the toasting tunnel. The acrylamide content of the toasted flakes was measured. Results are presented in Figure 9.2.

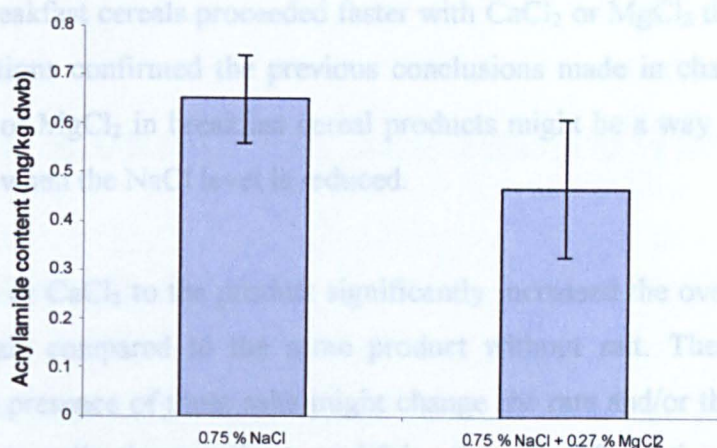


Figure 9.2: Acrylamide content of breakfast cereal products toasted for 1 min at 230 °C in the small scale toasting device.

The acrylamide content comparison between the products containing 0.75 % NaCl and with 0.75 % NaCl + 0.27 % MgCl₂ demonstrated that the addition of MgCl₂ did not significantly influence the acrylamide content ($p > 0.05$), even though the average content was decreased. This observation did not corroborate the results obtained by other authors. Indeed, a decrease of acrylamide content in bread by the addition of magnesium was previously observed (Sadd et al., 2008). Lower levels of acrylamide were found in a potato model system by the addition of CaCl₂ or MgCl₂ (Mestdagh et al., 2008b) and in fried potato chips when these were soaked in a solution of CaCl₂ (Ou et al., 2008). The absence of significant influence in this case might be due to the low level of MgCl₂ used or to the complexity of the food system compared to the model (composition and process used).

9.5. Conclusions

The main objective of this chapter was to observe the influence of CaCl₂ and MgCl₂ on the colour, taste and processability of breakfast cereals in order to conclude whether these salts could improve the product quality with a reduced NaCl content. Replacing completely the NaCl by 0.43 % CaCl₂ or MgCl₂ in the wheat and rice mixture cereals gave darker products than with 1.17 % NaCl. Hence, it seemed that within the concentration range studied, caramelisation reactions and/or starch

alteration in breakfast cereals proceeded faster with CaCl_2 or MgCl_2 than with NaCl . These observations confirmed the previous conclusions made in chapters 7 and 8. Adding CaCl_2 or MgCl_2 in breakfast cereal products might be a way to compensate for colour loss when the NaCl level is reduced.

Adding MgCl_2 or CaCl_2 to the product significantly increased the overall flavour of breakfast cereals compared to the same product without salt. These evaluations confirmed that presence of these salts might change the rate and/or the pathways of Maillard and caramelisation reactions, modifying the colour formation in the product but also the overall flavour molecules generated. Hence, adding MgCl_2 or CaCl_2 could improve the taste of the products when a lower level of NaCl is used. However, it should be noted that even though CaCl_2 or MgCl_2 increased the overall flavour of breakfast cereals compared to a product without any salt, the overall and toasted flavours were still considered significantly stronger with the standard level of NaCl .

The average acrylamide content of the products was decreased by the addition of 0.27 % of MgCl_2 when the product contained 0.75 % NaCl .

The addition of CaCl_2 or MgCl_2 to the breakfast cereals might improve the overall flavour, colour and acrylamide content of the products. Indeed, these salts might compensate for some of the influence of NaCl on starch degradation and on Maillard and caramelisation reactions, which are the two reactions greatly influencing the quality of these food products.

10. Conclusion and future work

10.1. Main findings

The average NaCl daily intake in the UK has reached 9 to 12 g per person, which is twice as much as the medical recommendations. As around 75 % of the sodium consumed comes from processed foods, food industries are encouraged to reduce the NaCl level in their products. The Food Standard Agency in the UK has set a target level as less than 0.8 % of NaCl in breakfast cereals. This level is much lower than is currently present in some products found on the market. In order to decrease the NaCl concentration of breakfast cereals without altering the product properties, such as its taste and colour, a better understanding of the role of NaCl in the product was needed.

In order to define the impact of NaCl on certain product characteristics, a model system was developed. This system, composed of native starch, glucose and a cocktail of amino-acids, mimicked breakfast cereals during the toasting stage in terms of colour development and the most intense residual volatile molecules released. This model was used to observe the impact of NaCl on these attributes. Adding asparagine to the amino-acid cocktail allowed the study of the influence of NaCl on acrylamide formation.

By the use of this model system and several breakfast cereal products, it was found that:

- NaCl significantly influenced colour formation. When NaCl was added to the model systems or breakfast cereal, darker products were obtained after the toasting stage. This colour enhancement was proportional to the salt concentration.

- NaCl significantly decreased acrylamide formation in models and breakfast cereal products.
- The NaCl level did not influence the most intense residual volatile molecules released from breakfast cereals. Different results were obtained concerning the impact of NaCl on these volatiles in model systems, depending on the pH and composition of the models. However, when the model parameters (pH and composition) were the closest to breakfast cereals, similar results to the food products were then obtained.

These observations made on model systems and breakfast cereal products concerning colour and acrylamide formation seemed to indicate a possible impact of NaCl on Maillard and/or caramelisation reactions. Investigations were then carried to demonstrate whether it was due to the plasticising effect of NaCl on the system, keeping the product longer in a rubbery state. Another hypothesis was that water might remain longer in the product during the toasting stage as NaCl is a hygroscopic compound. In both cases, molecular mobility could be improved, facilitating Maillard reactions. However, it was found that neither of these hypotheses could explain the influence of NaCl on colour formation. It was found that presence of NaCl did not significantly influence moisture retention and the glass transition temperature of the system was not correlated to the colour formation during a heat treatment.

Molecular mobility is critical mainly for Maillard reactions as bimolecular condensation steps take place at an early stage of the reaction. The absence of correlation between colour formation and the T_g of the system might indicate that colour formation could be mainly due to caramelisation reactions, where molecule mobility is not as critical. In order to better understand how NaCl was influencing colour and acrylamide formation, simpler model systems were needed. The model system developed earlier was then broken down in order to explore the influence of NaCl on starch integrity during a heat treatment, and observe separately how NaCl impacts Maillard reactions and caramelisation reactions. It was found that:

- During a heat treatment mimicking the toasting stage, presence of NaCl significantly enhanced native starch granule disruption and starch polymer degradation. This might indicate that the starch of breakfast cereal products could be degraded during the toasting stage. Such degradation could then generate glucose, which could caramelise and lead to colour formation. These observations might explain why darker breakfast cereal products were obtained when NaCl was present in the composition.
- Presence of NaCl significantly increased colour formation during the caramelisation reactions of glucose. If presence of NaCl promotes at the same time starch alteration and glucose formation during the toasting stage of breakfast cereals, colour enhancement could then be explained by both the higher amount of glucose taking part in the caramelisation reactions (due to starch degradation) and the catalysing action of NaCl on caramelisation.
- Presence of NaCl in a system composed of glucose and amino-acids led to significantly lighter products when these systems were heat treated in a similar way as occurs during the toasting stage. Hence, this might indicate that NaCl could slow down Maillard reactions under the conditions investigated. Such an impact of NaCl on Maillard reactions could explain why acrylamide formation was decreased proportionally to the amount of NaCl present in breakfast cereals.

Therefore, in breakfast cereal products, both Maillard and caramelisation reactions seem to happen at the same time during the toasting stage. Caramelisation reactions might overcome Maillard reactions regarding colour formation. Indeed, darker breakfast cereal products were obtained when NaCl was present, although colour formation via Maillard reactions seemed to be inhibited by the presence of NaCl. Decreased acrylamide formation in breakfast cereals when NaCl was present indicated that Maillard reactions were also taking place during the toasting stage of breakfast cereals and were slowed by NaCl.

This study demonstrated that NaCl could enhance starch degradation during a severe heat treatment such as that occurring during the toasting stage. At the same time, it

promoted colour formation via caramelisation reactions. However, one phenomenon could be the consequence of the other. Indeed, caramelisation reactions lead to the formation of H^+ . Acidic products are known to catalyse starch degradation during roasting (starch pyroconversion). Therefore, during the toasting stage, the starch molecules could be degraded by the heat applied to the product, leading to some glucose formation. Caramelisation might then take place, enhanced by the presence of NaCl. Acidic products might then be generated, promoting starch degradation and glucose formation. Hence, presence of NaCl might only indirectly have an impact on starch during a heat treatment via its influence on caramelisation reactions leading to acidic product formation.

It was found in Chapter 6 that presence of plasticisers (NaCl, KCl or trehalose) significantly enhanced colour formation of model systems containing pregelatinised starch, glucose and amino-acids compared to the same models without plasticiser (Figure 6.6). According to the results in Chapter 7 and 8, NaCl and KCl should enhance colour formation via caramelisation (overcoming the inhibition of colour formation via Maillard reactions). This explains the colour differences between the systems with and without plasticisers. It should be noted that in those systems, as amino-acids were present, the pH of the system was low. Hence, the pH decrease due to caramelisation reactions during the heat treatment should be minor, as the pH of the system was already around 3.5. If it is considered that the pH change due to caramelisation reactions in systems containing starch, amino-acids and glucose was negligible, the colour differences between the models with and without plasticisers should only be due to the presence of plasticisers. It then seems to indicate that these plasticisers helped starch degradation, generating smaller molecules like glucose and increasing the caramelisation reactions extent (hence colour). The hypothesis that starch degradation during a heat treatment might be only the consequence of the lower pH obtained due to caramelisation reactions is not valid anymore if the pH change is considered negligible. However, to confirm such statement, more work in that area is needed.

NaCl seemed to influence Maillard and caramelisation reactions. These two types of reactions are known to generate aroma and flavour molecules. Therefore, decreasing

or removing NaCl from breakfast cereal recipes might not only alter the salty taste of these products but also the overall flavour.

Other types of salts, such as CaCl_2 or MgCl_2 , were used in this study to observe their impact on native starch degradation and colour formation via caramelisation and Maillard reactions during a heat treatment. It was found that both these salts had a similar (or even more efficient) impact as NaCl on these reactions. Therefore, if decreasing or removing NaCl from breakfast cereals affects the overall flavours generated via Maillard and caramelisation reactions, adding CaCl_2 or MgCl_2 to the recipe might compensate for a potential flavour alteration.

These salts (CaCl_2 or MgCl_2) were added to breakfast cereals as a partial or total replacement of NaCl. It was found that adding these salts compensated for colour loss due to the NaCl decrease. The overall flavour of breakfast cereals containing CaCl_2 or MgCl_2 increased compared to the recipes without any salt. This seemed to indicate that NaCl, as well as these 2 other salts, indeed influenced flavour generation and/or release in breakfast cereals. However, it should be noted that the addition of CaCl_2 or MgCl_2 did not restore entirely the product flavour alteration due to a NaCl reduction based on the 7 flavour attributes tested in this study (overall, toasted, salty, sweet, bitter, drying and off flavours). Moreover, the salty taste of the breakfast cereals was not increased by the presence of CaCl_2 or MgCl_2 compared to the recipes without any salt.

The influence of salts during the toasting stage of breakfast cereal products can be summarised as represented in Figure 10.1.

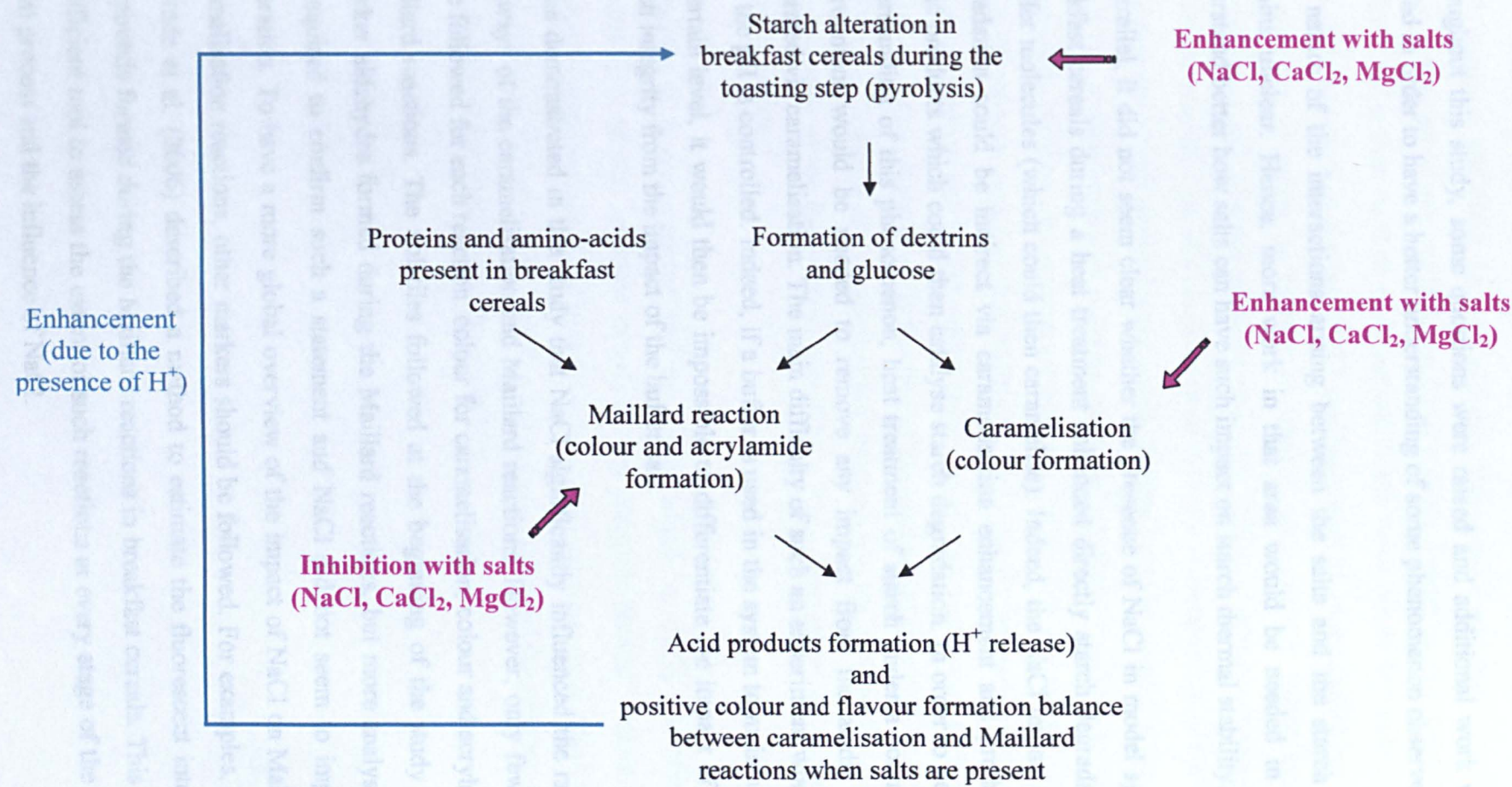


Figure 10.1: Influence of NaCl, CaCl₂ and MgCl₂ on colour, acrylamide and flavour formation during the toasting step of breakfast cereal products.

10.2. Future work

Throughout this study, some questions were raised and additional work would be needed in order to have a better understanding of some phenomenon observed.

The nature of the interactions arising between the salts and the starch polymer remains unclear. Hence, more work in that area would be needed in order to understand better how salts can have such impact on starch thermal stability.

In parallel, it did not seem clear whether the presence of NaCl in model systems or breakfast cereals during a heat treatment enhanced directly starch degradation into smaller molecules (which could then caramelize). Indeed, the NaCl impact on starch degradation could be indirect via caramelisation enhancement and production of acidic products which could then catalyse starch degradation. In order to get a better understanding of this phenomenon, heat treatment of starch under a controlled pH environment would be needed to remove any impact from the acidic products generated via caramelisation. The main difficulty of such an experiment would be the way the pH is controlled. Indeed, if a buffer is used in the system to maintain the pH to certain level, it would then be impossible to differentiate the impact of NaCl on starch integrity from the impact of the buffer salt.

It was demonstrated in this study that NaCl significantly influenced the rate and/or pathways of the caramelisation and Maillard reactions. However, only few markers were followed for each reaction: colour for caramelisation, colour and acrylamide for Maillard reactions. The volatiles followed at the beginning of the study could be Strecker aldehydes formed during the Maillard reactions, but more analyses would be required to confirm such a statement and NaCl did not seem to impact their generation. To have a more global overview of the impact of NaCl on Maillard and caramelisation reactions, other markers should be followed. For examples, Delgado-Andrade et al. (2006) described a method to estimate the fluorescent intermediate compounds formed during the Maillard reactions in breakfast cereals. This might be an efficient tool to assess the extent of such reactions at every stage of the breakfast cereal process and the influence of NaCl.

In Chapter 8, the influence of NaCl on Maillard and caramelisation reactions (via colour formation) was studied. The compositions of the systems used were relatively simple (glucose and NaCl for one, glucose, amino-acids and NaCl for the other). Hence, it seemed to indicate that NaCl had a direct impact on the chemistry of the reactions rather than on their environment. Some work would be needed to have a better insight regarding the way NaCl can have such an impact on those reactions. For example, it could be helpful to identify and quantify some intermediate compounds of each of those reactions versus the NaCl concentration present. Indeed, NaCl could play the role of a catalyser or an inhibitor at certain steps of the reactions, modifying the overall molecules generated. It should also be noted that the influence of NaCl on Maillard reactions could be due to its interactions with the proteins or the amino-acids. As those compounds are charged, the sodium and chloride ions could screen the charges, changing their reactivity. NaCl could also modify their solubility (salting in / salting out effect), which could change the way the proteins or amino-acids are reacting. A better understanding of the mechanism by which NaCl is having an impact on Maillard and caramelisation reactions is critical. Indeed, it would help restoring some of the flavour loss caused by a NaCl decrease in the formulation of breakfast cereals.

In Chapter 9, it was observed that the use of MgCl_2 or CaCl_2 was affecting the processability of the breakfast cereals as the products became stickier. Such an influence should be understood in order to be able to use other types of salt to replace NaCl in cereal products. An interaction between the salts and the proteins and/or the salts and the starch should again be envisaged.

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